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STUDIES ON THE BIOLOGY OF THE FROG AMPHISTOME, *DIPLODISCUS TEMPERATUS* STAFFORD[1]

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Diplodiscus temperatus Stafford, a rectal fluke parasite, infects both tadpoles and frogs. Krull and Price (1932) found that this trematode may either infect the tadpole and develop to the stage where it can shed eggs, or it may grow for some time and remain in the immature condition in some part of the alimentary canal of the tadpole. It then matures after the tadpole metamorphoses into a frog. They also noted that during metamorphosis the flukes migrate "to all parts of the intestines as far forward as the stomach." In their report is found the first conclusive, experimentally-determined life history of this species. Looss (1892), however, published the complete life history of *Diplodiscus subclavatus* (syn. *Amphistomum subclavatum*), in which he described all the stages. He found the mother sporocyst and one generation of rediae. Krull and Price found no mother sporocyst and also no mother rediae. In a recent paper (Herber, 1938), a mother redia was reported for *D. temperatus*. Krull and Price stated that frogs are usually infected by eating their own stratum corneum containing encysted metacercariae of this species. Tadpoles are infected by eating encysted metacercariae in the pond ooze or by taking the active cercariae into the mouth during respiration. Excystment occurs in the small intestine and most flukes are found in the rectum. The eggs are shed there and passed out with the feces. The miracidium escapes shortly afterwards and infects the snail, *Helisoma trivolvis* (Say). After some time cercariae escape from the snails and encyst as metacercariae.

Studies on *D. temperatus* are here extended to include a study of the distribution of flukes in all parts of the intestine in natural and experimental infections. Studies on infections in very young hosts and in very heavy exposures are also included.

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[1] Contribution from the University of Michigan Biological Station. I wish to take this opportunity to express my appreciation to Dr. W. W. Cort under whose direction these studies were made, for his suggestions and criticisms, and to Dr. L. J. Thomas for his many practical suggestions.

HOST MATERIAL

The leopard frog, *Rana pipiens* (Latreille), and the snail, *H. trivolvis*, were the prinicpal hosts used. *Helisoma antrosum percarinatum* (Walker) and *Helisoma campanulatum smithii* (Baker) are reported as additional intermediate hosts. For the study of natural infections, frogs, tadpoles, and snails were collected from the Bessey Creek and Fontinalis Run areas of the Douglas Lake region. Frogs and tadpoles used as experimental hosts were taken from Vincent Lake, a small acid lake, in which snails do not occur and from which frogs infected with rectal flukes have never been taken.

Mature frogs could be kept as long as needed at a cool place in an aquarium padded with wet sphagnum. Sometimes grasshoppers were used as food for the frogs. Tadpoles were distributed in lots of 16–20 in large glass culture dishes. About a pint of well water was added to these vessels which were slightly tilted so that the tadpoles could come out of the water. Unless otherwise mentioned, experimental tadpoles were fed lettuce trimmings and the water was changed daily. Snails were collected from shallow water habitats and kept in pint milk bottles. The snails were fed bits of lettuce leaves and the water was changed daily.

BIOLOGY OF MATURE AND IMMATURE ADULTS

Natural infection. The observations reported here were made during the latter part of June, all of July, and the early part of August. During the summer of 1935, 153 tadpoles collected from the areas of natural infection showed 98 per cent infection. During the same season, 16 adult frogs showed 63 per cent infection. Only infected hosts are here considered because this part of the study is confined to the distribution of the flukes. Most of the flukes found in tadpoles were immature, although a few contained eggs in the uterus. The average number of flukes in the tadpoles was 18 while in frogs the average was only 4. Sexually mature flukes were usually found in adult frogs during the late summer.

Distribution of flukes in natural infections in the digestive tract of tadpoles and frogs of different ages. Tadpoles and frogs were collected in lots of about 30 from heavily infected areas. They were examined at autopsy by removing the entire alimentary canal and dividing it into four parts consisting of stomach, upper half of intestine, lower half of intestine and rectum. The individual parts were then washed in watch glasses and a count of the flukes made.

Table 1 presents data on the distribution of flukes in the digestive tract of frogs and tadpoles of different ages. Since the ages were not known, lengths of the alimentary canal were used to separate the animals according to their stage of development.

TABLE 1.—*Distribution of flukes in the digestive tract of tadpoles and frogs in different stages of development*

Length alimentary canal	Frog hosts		Number of flukes in							
			Stomach		Upper intestine		Lower intestine		Rectum	
Inches	Number	Extent metamorphosis	Median	Range	Median	Range	Median	Range	Median	Range
11–20	21	2–leg tadpole							10	1–52
9–10	13	2–4 " "					1	0–13	6	2–13
7– 8	10	2–4 " "			1	0–7	3	0–16	6	2– 8
5– 6	6	2–4 " "	1	0–2	2	0–5	1	0– 5	8	2–14
3– 4	12	27mm tail "	5	0–36	1	0–34	1	0–10	6	0–29
1– 2	39	18mm tail "	3	0–32	1	0–10	1	0–14	1	0–16
1	35	small frog			0	0–10	0	0– 5	5	0–21
3–11	14	adult frog							2	0–37

TABLE 2.—*Position of flukes in transforming tadpoles of different ages after thyroxin treatment*

Length alimentary canal	Frog hosts		Number of flukes in							
			Stomach		Upper intestine		Lower intestine		Rectum	
Inches	Number	Extent metamorphosis	Median	Range	Median	Range	Median	Range	Median	Range
8–10	2	2–legs	1	0– 1	1	0– 1				
6– 7	4	2–4 legs	1	0– 6	25	1–77	3	1– 5	1	1– 2
1– 4	35	2–4 legs	7	0–24	1	0– 8	0	0– 3	7	0–11
									2	0–20

This table shows that in mature frogs and young tadpoles the flukes are usually found in the rectum. The number of flukes found in these natural infections varied from none to 52. In tadpoles with an alimentary canal length of about 8 inches some flukes were found in the upper and lower halves of the intestines. When the length is about 4 inches, many flukes are found in the stomach. As the tadpole completes metamorphosis, flukes are no longer found in the stomach.

These data indicate that the flukes migrated forward from the rectum as the intestine shortened. Possibly they begin their migration in search of certain food environments, since a large amount of food is expelled from the gut when it shortens rapidly. At the time they are found in the stomach, some food is found there and very little in any other part of the digestive tract. It is evident that this migration occurred after these parasites had been established in the rectum, since Krull and Price found that in 24 hours after infection most of the young amphistomes were in the rectum. These migrating flukes were found in all stages of development, with several sexually mature in the stomachs of the tadpoles.

At the time the frog type of gut is formed and insects are taken into stomach, the parasites appear to migrate back to the rectum or are lost. In 37 newly metamorphosed frogs only 1 fluke was found in the upper intestines and only 2 frogs had any of them in the lower intestines. Also all the mature frogs harbored all their amphistomes in the rectum, the number varying from 1 to 37. This migration back to the rectum is so definitely shown in these observations that an attempt was made to discover differences in the stomach contents of the metamorphosing tadpole and the frog.

It is well known that frogs eat insects and other protein food which necessitates an acid condition for its digestion. Migration of the flukes back to the rectum might be caused by their having a negative reaction to a stomach acid enough to digest insects. To test this hypothesis hydrogen ion determinations (quinhydrone) were made of separate parts of the alimentary canal and correlated with the number of parasites found.

In 10 recently metamorphosed frogs, although flukes were found in the recta, none were in the stomachs. Stomach contents when mixed with 20 cc of distilled water had a pH of 5.2, the rectal contents in a similar mixture a pH of 7.1. A second similar series of 5 unparasitized stomachs had a pH of 5.2, the contents of the recta (parasitized) a pH of 7.2. On the other hand, using metamorphosing tadpoles, the pH of contents of 8 parasitized stomachs was 7.2, and that of the contents of the recta (parasitized) was 7.5. Thus flukes were not found in acid environments which suggests that when the contents of the tadpole stomach changes from alkaline to acid, they find it an unfavorable environment and leave.

In order to study the effects of metamorphosis on this migration, thyroxin was administered to several groups of naturally infected tadpoles. Table 2 shows the data on the position of flukes in the digestive tract of naturally infected tadpoles after treatment with 1:250,000 thyroxin for varying periods. After 5 days exposure to daily changes of thyroxin the tadpoles became thin and began to show evidences of metamorphosis externally. Legs were formed underneath the skin but failed to perforate it, making their appearance somewhat irregularly. Apparently the thyroxin also stimulated the alimentary canal to empty its contents more rapidly than in the untreated group. Some of the tadpoles were autopsied before the intestines had shortened very much while others were kept in the solution until metamorphosis was almost complete. This gave comparative data on tadpoles which metamorphosed rapidly by thyroxin treatment (Table 2) and those which metamorphosed naturally (Table 1). It is seen that while the number of flukes is about the same in the treated and untreated frogs some of them are found in the stomachs of thyroid-treated tadpoles with the longer alimentary canal lengths.

These observations show that in young tadpoles and mature frogs the flukes are usually found in the rectum. During metamorphosis of the tadpole they disperse towards the stomach. Metamorphosis of tadpoles is associated with a complete change in the structure of the alimentary canal. The stomach wall becomes thicker, the intestines and rectum become straight and short. The entire length of the alimentary canal is reduced from over 10 inches in the tadpole to one inch in the newly metamorphosed frog. This change is accompanied by a greatly reduced amount of alimentary canal contents. Scarcity of food in these animals seems to initiate a dispersal of flukes from the rectum towards the stomach. Their movement away from the stomach of the newly metamorphosed frog is probably a negative response to the acid condition there. In thyroxin-treated tadpoles the alimentary canal seems to be stimulated to release its contents rapidly. This is probably the reason why more flukes were present in the stomach of thyroid-treated tadpoles when the entire gut had shortened very little.

Experimental infection in very young tadpoles. An attempt was made to find out on which day in the early life of the tadpole it would first become infected. Some eggs of *Hyla crucifer* (Wied) were brought into the laboratory on June 27, 1935. Upon examination they were found to have developed to the gastrula stage. External gills disappeared on July 7th and the mouth appeared on July 9th. About a dozen of these tadpoles were isolated in a finger bowl to which many cercariae of *D. temperatus* were added. On July 10th 3 flukes were found in the rectum of one of the tadpoles and 12 in another tadpole. The remainder of the tadpoles were kept without any further attempt at reinfection to

see if the flukes would develop to maturity. Flukes were still found on July 12th but at the end of a month all had been lost in 3 surviving tadpoles. This experiment shows that the tadpoles of *H. crucifer* can be infected 13 days after the gastrula stage of egg development.

Since the exact time of fertilization of the eggs was not known for the above experiment, ovulation was induced in a female *R. clamitans* (Latreille) by the Rugh pituitary implant technique. On July 9th at 6:00 PM, eggs were stripped from the frog into a dish having a shallow layer of water over the bottom, and crushed testes spread over these eggs. First segmentation was observed at 7:30 PM. The developing embryos were kept at an average temperature of about 23° C, and open mouth forms were first seen on the 10th day. Infection was attempted the same day by isolating a dozen tadpoles in a finger bowl and exposing them to many cercariae. One tadpole, autopsied within 24 hours, was found parasitized. It is evident that tadpoles of *R. clamitans* can be infected 10 days after they begin development. So far as known this is the earliest fluke parasitism recorded for frogs. Tadpoles examined from the infected batch 2 weeks later showed no fluke parasitism.

Heavy experimental infection. Two groups of tadpoles were infected daily to determine the highest number of flukes capable of crowding into the rectum. In the first group 12 tadpoles of *R. pipiens* were exposed daily for 6 days to the cercarial output of 9 snails. The tadpoles had developed during the spring up to the stage where a few had forelegs. The number of cercariae escaping from the snails varies up to about 300 per day, the number depending upon the temperature, the age of the infection, and the length of time the snails were exposed to laboratory conditions. The average number of cercariae given off by a medium-sized snail under laboratory conditions is about 50 per day. The tadpoles were autopsied at intervals of 3 to 20 days after the beginning of the infection. Eight days after the end of the infection period the tadpoles began to lose the flukes rapidly. The highest number of flukes found in any tadpole was 252 which was recorded from an autopsy of 7 days after the end of the infection period. The lowest number recorded was 7 from a tadpole 15 days after the last exposure to cercariae. The average number of flukes recovered from these tadpoles was 48.

The second group of 8 tadpoles of *H. crucifer,* almost ready to metamorphose, was exposed to all the cercariae which escaped in one day from 8 snails. The tadpoles were examined one to 12 days after the infection. The highest number recovered was 112 from the rectum of a tadpole on the second day after the exposure to the cercariae. The average number of flukes recovered was 35 and one tadpole harbored none at all.

Of all the 860 flukes found in the two groups, only 23 were found outside the rectum and these were recovered from tadpoles which were in the later stages of metamorphosis. In fact, no matter how heavy the infection, the parasites were found in the recta of young tadpoles. This indicates that the migration of the flukes from the rectum is not induced by heavy infections in young tadpoles as implied by Krull and Price.

SUMMARY

Observations were made on the biology of *D. temperatus* (from both natural and experimental infections) in a region where heavy natural infections are present in *R. pipiens* and *R. clamitans,* with *H. trivolvis* as the intermediate host. *H. antrosum percarinatum* and *H. campanulatum smithii* are reported as additional intermediate hosts.

The rectum was found to be the normal site for these flukes in young tadpoles and frogs but during metamorphosis of the tadpole the flukes migrate away from the rectum toward the stomach. When the young frog begins to eat insects the flukes migrate back towards the rectum and no flukes were found in the acid stomach of the insect feeding frog. When infected tadpoles were treated with thyroxin dispersal of flukes occurred in early stages of metamorphosis apparently because of heightened peristalsis. Changes in the amount and type of food seem to be the main reason for the distribution of flukes in the digestive tract of metamorphosing tadpoles.

Tadpoles of *R. clamitans* can be infected when they are 10 days old or as soon as the mouth opens. The heaviest experimental infection in tadpoles of *R. pipiens* was 252 and in those of *H. crucifer,* 112. In each case all the flukes were found in the rectum.

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ON THE MORPHOLOGY OF *BALANTIDIUM KIRBYI* N. SP., FROM THE PLATHANDER

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Since the establishment of the genus *Balantidium* by Claparède and Lachmann in 1858 for a ciliate which was first observed by Ehrenberg (1838) in the rectum of frogs and which he had named *Bursaria entozoon,* there have been described a large number of species from many animals. These have been found in the higher vertebrates such as man and monkey, and more recently in camels (Hegner, 1934) and the ostrich (Hegner, 1934). They are, however, most common in the lower vertebrates, especially frogs and toads.

The species described in this paper was encountered in the South African Plathander, *Xenopus laevis* (Daudin), and is named *Balantidium kirbyi* in honor of the man who first observed it.

MATERIAL AND METHODS

Balantidium kirbyi occurs in great numbers in the large intestine of *Xenopus laevis* from Cape Province, South Africa. Prepared slides fixed in Hollande's fluid and Schaudinn's fluid and stained in Heidenhain's and Delafield's hematoxylin were used.

In order to study the morphology in detail, intestines were fixed in Bouin's fluid, cut into sections 7 μ thick, and stained in Heidenhain's hematoxylin. Differentiation was most successful with Tuan's method of destaining with a saturated aqueous solution of picric acid instead of iron alum. This was especially noticeable in the differentiation of the intestinal wall.

In cross-sections the organisms were found in the lumen of the gut, and no case of tissue invasion was seen.

Balantidium kirbyi n. sp.

(Figs. 1–6)

Specific diagnosis: Genus *Balantidium.* Body of organism typically elongated with anterior end rounded and posterior tapering and roughly pointed (Fig. 1). Covered with longitudinal rows of cilia that gradually curve as they proceed posteriorly.

Based on 100 measurements (following, with modifications, Hegner, 1934), length of body is 275 μ (149–345) and breadth 78 μ (44–110). Ratio of length to breadth 3.5 : 1.

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* I wish to express my appreciation to Dr. Harold Kirby, Jr., who provided me with prepared slides, and to Professor C. de Villiers, of Stellenbosch, South Africa, for additional material. The author also wishes to thank Mr. John Mohr for his many thoughtful suggestions which were a great aid in the course of this work.

Macronucleus and Micronucleus. Characteristic position of macronucleus is in posterior third of body, although also found in anterior two-thirds (Fig. 1). Macronucleus typically kidney or bean-shaped, circular in section, and lies normally with long axis coinciding with long axis of the *Balantidium* (Fig. 1). Length of macronucleus 51 μ (32–64) and breadth 19 μ (12–26). Ratio of length to breadth 2.5: 1. Micronucleus in an eccentrically placed depression in macronucleus and in sagittal section is biconvex in shape and in cross-section is oval or circular. Length of micronucleus 2 μ (1–3) and of cytopyge 15 μ (13–26). Macronucleus in slightly stained specimens has a uniformly granular appearance, while micronucleus in the same specimens is consistently more lightly stained and more finely granular. A membrane attaches the micronucleus to the macronucleus.

Basal Apparatus. Cilia perforate the pellicle and are attached to basal granules which lie directly underneath. Granules inter-connected by fibrils. Ciliary rootlets extend from these granules centrally into endoplasm where they are attached to other granules. These deeper granules appear to take a heavier stain than basal granules and are also connected to each other by fibrils.

Cytoplasm. Food vacuoles and granules are uniformly distributed throughout endoplasm, anteriorly as well as posteriorly. A thin ectoplasm below pellicle appears more heavily granulated than endoplasm.

Excretory System. Excretory vacuoles are present in varying numbers, usually 3 (1–5). Position of these vacuoles varies, but generally two are present in endoplasm close to periphery of caudal third of organism, and the third in vicinity of periphery of anterior two-thirds of body. A cytopyge is present in all specimens at posterior end, but only in two specimens have there been seen vacuoles emptying into it.

Peristome and Cytopharynx. Peristome begins anteriorly towards right margin on ventral side and often extends for about one-fourth of length of body. Its anterior end is broader than its posterior end. Adoral row of long cilia is present which delimits peristome and then continues in spiral manner through cytopharynx to its end. Thick row of cilia (membranelles) is found below long cilia, originating on left of peristome, close to anterior part of the body.

Dorsal Spines. On dorsal side of anterior third of animal occurs a group of spines which vary in length from 20 to 50 μ. Area covered is on diagonal from left anterior dorsal side to right dorsal side for about one-third of length of the animal (Fig. 2). Spines sparse in area of origin, gradually increasing in density and slightly in length until reaching the approximate center where they are rather congested, and then thin out again posteriorly. Anterior thin ends are imbedded in cytoplasm while thicker posterior ends appear to lie on pellicle.

Fibrillar System. System of fibers consisting of four parts: (1) distal elongated basal rod composed of several fibers, located close to the surface on ventral side and on level with ventral posterior end of peristome; (2) a group of heavy fibers arising from anterior end of basal rod and radiating anteriorly, ending abruptly close to surface; (3) a group of more delicate branching fibers which also arise from anterior end of basal rod and are distributed to anterior end of organism, but which are situated deeper in cytoplasm; (4) a single set of fibers which pass directly from basal rod diagonally and slightly posteriorly to surface, and appear to end in deeper row of granules (Fig. 3).

Host: Xenopus laevis.

Location: Large intestine.

Locality: Cape Province, South Africa.

The general body outline is somewhat like that of *B. sushilii* Ray, 1932, which occurs in *Rana tigrina* from Calcutta. Table 1 shows how this species differs from the one described by Ray.

There is a similarity between the basal apparatus system described in this paper and that described by McDonald (1922), but I have been un-

TABLE 1

	B. sushilii	*B. kirbyi*
Size	150–319 × 35–65 μ	149–345 × 44–110 μ
Shape	Torpedo-shaped	Torpedo-shaped
Macronucleus	Broadly oval	Bean-shaped
Position of macronucleus	Very variable	Posterior third of body
Position of micronucleus	Lateral to the macronucleus	Lateral to macronucleus, usually off-center
Contractile vacuoles	Three; two lateral and one terminal	Number and position vary; 1–5, usually 3
Borer	Present	Absent
Dorsal spines	Absent	Present
Fibers	On margins of peristomal lips	None present near peristome
Cytopharynx	Not described	Conspicuous, long and curved posteriorly

able to find the granular and hyaline bands below the pellicle that he described.

A characteristic of this species of *Balantidium* that differentiates it from others is the length and shape of the cytopharynx. It forms a sigmoid curve from the peristome and then at its posterior end is strongly recurved. The length of the cytopharynx is, on the average, half of the length of the body. In *B. sushilii*, Ray described a comparable peristome, but did not mention a cytopharynx. In three cases balantidia were seen with nucleated cells and other dark-staining bodies in the cytopharynx.

It was found that the spines were best demonstrated by Schaudinn's fixative and iron hematoxylin.

SUMMARY

1. *Balantidium kirbyi* n. sp. is described from *Xenopus laevis* of Cape Province, South Africa.

2. The shape and length of the cytopharynx in *B. kirbyi* is unique in this genus. It has a length on the average of one-half of that of the body and in shape, starting from the peristome, is a sigmoid curve which at its posterior end strongly recurves.

3. The presence of a group of spines on the anterior dorsal surface of the animal has been described in no other species of *Balantidium*.

4. The fibrillar system consists of a basal rod close to the ventral surface on a level with the posterior end of the peristome, from which three other groups of fibers arise. One group of heavy fibers radiates anteriorly, a larger group of delicate branching fibers below these also pass anteriorly, and a single set of fibers passes to the surface.

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EXPLANATION OF PLATE

All drawings made with the aid of a camera lucida.

FIG. 1. Drawing of whole animal showing characteristic shape and length of cytopharynx and position of nuclei and excretory vacuoles. ×455.

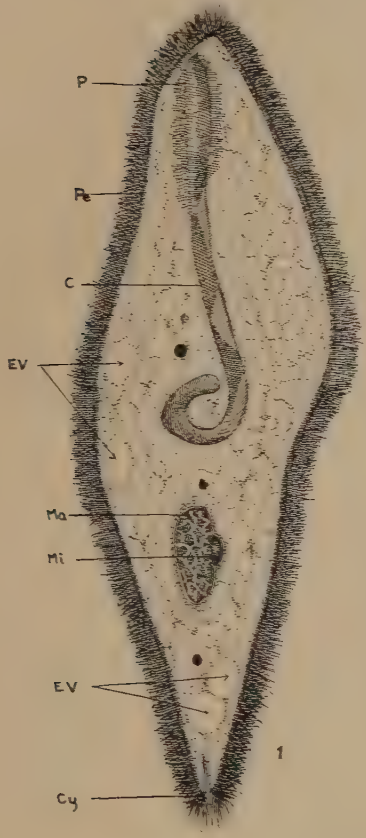
FIG. 2. Detail of anterior spines, dorsal view. Schaudinn's fixative and hematoxylin stain bring the spines into prominence. ×455.

FIG. 3. Fibrillar system showing its four components. Note lack of fibers in vicinity of peristome. ×455.

FIGS. 4–6. Various shapes of body showing position of peristome and macronucleus and cytopharynx. ×218.

ABBREVIATIONS USED

P—Peristome
Pe—Pellicle
C—Cytopharynx
EV—Excretory vacuoles
Ma—Macronucleus
Mi—Micronucleus
Cy—Cytopyge
ADF—Anterior delicate fibers
HF—Heavy fibers
FS—Fibers to surface
BR—Basal rod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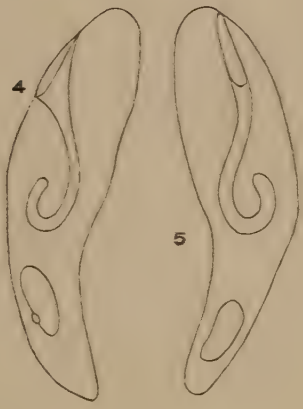
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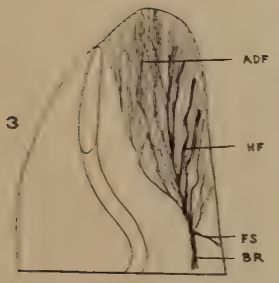
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A NEW TAPEWORM, *CHOANOTAENIA IOLA*, FROM THE ROBIN[1]

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In a robin, *Planesticus migratorius migratorius* (Linn.), taken at Champaign, Illinois, in June, 1936, the writer encountered approximately 75 specimens of a tapeworm. It was demonstrated that these cestodes represent a species previously unrecognized in the literature, and that they belong to the genus *Choanotaenia* Railliet 1896, although possessing a character which violates the current concepts of the genus and of the family *Dilepididae*.

The name *Choanotaenia iola*, is proposed, and a description of the species is given together with a redefinition of the family *Dilepididae* and the genus *Choanotaenia*.

Choanotaenia iola n. sp.

(Figs. 1–4; Table 1)

Specific diagnosis: Genus *Choanotaenia* Railliet, 1896. Small cestodes, average length about 2.4 mm, maximum width 557 μ. Anterior proglottids broader than long, with average maximum breadth at anterior end 424 μ. Approximately 21 segments present in strobila, with one or two gravid. Mature segments about as long as broad, gravid ones longer than wide. Proglottids telescoped, overlapping. Genital pores irregularly alternating, occasional strobila showing regularly alternating pores.

Scolex prominent, average width 356 μ with four cup-shaped suckers. Average width of suckers 114 μ with inner surface armed throughout with cuticular spines 3–5 μ long (Fig. 4). Rostellum bears crown of 17–20 hooks (Figs. 1 and 3). Average width of crown and body of rostellum 68 and 65 μ respectively. Rostellar hooks approximately 32 μ long. Muscular sac 202 μ long.

Female genital complex. Vagina in ventral position along anterior half of proglottid margin, and dilated at distal end near center of proglottid forming receptaculum seminis. Ovary 36 μ in diameter. Oviduct arises dorsally receiving first the vitelline duct, secondly, the spermatic duct from receptaculum seminis. Vitelline gland lobated, pyramidal in outline, apex directed posteriorly, greatest width 63 μ at base. Union of oviduct and spermatic duct forms oötype into which shell gland empties. Uterus, a laterally branching organ, arises on poral and antiporal sides of oötype at approximately the level of shell gland. Uterus ultimately breaks up into individual capsules.

Male genitalia. Testes 13–17 in number posterior and lateral to female complex not confined to single plane, but scattered throughout depths of proglottid. Average diameter of testes 50 μ. Vas deferens coiled before entering cirrus pouch. Cirrus heavily armed with minute spines.

Host: Planesticus migratorius migratorius (Linn.).

Habitat: Small intestine.

Locality: Champaign, Illinois.

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[1] Contribution from the Zoological Laboratory, University of Illinois, No. 528.

[2] The writer wishes to express his appreciation to Dr. H. J. Van Cleave, under whose direction this work was carried out.

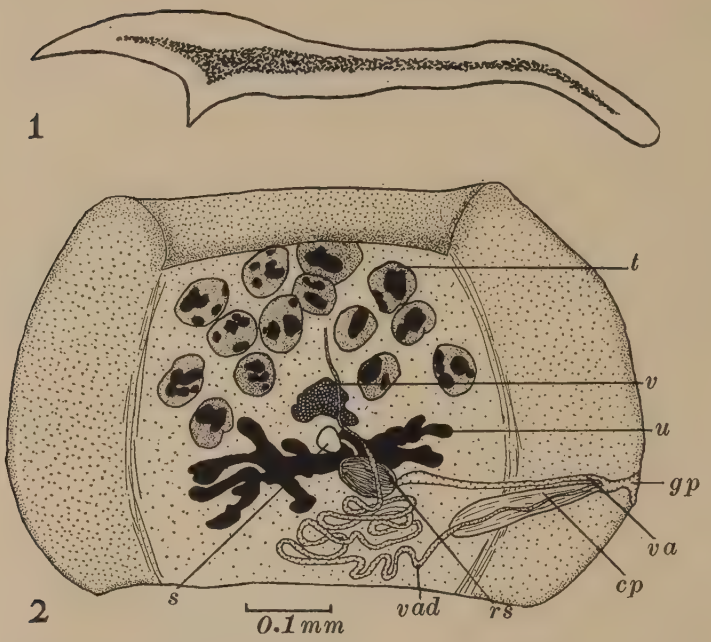

Fig. 1, rostellar hook (free-hand drawing) and, Fig. 2, mature proglottid of *Choanotaenia iola* n. sp.

Abbreviations: t—testis, v—vitellarium, u—uterus, gp—genital pore, va—vagina, cp—cirrus pouch, rs—receptaculum seminis, vad—vas deferens, s—shell gland.

Holotype: U. S. Nat. Mus., No. 9230. Paratypes in collections of H. J. Van Cleave at Urbana, Illinois, and of the writer.

Choanotaenia iola n. sp. differs from all other members of the genus with the exception of *Choanotaenia infundibulum* (Bloch, 1779) in the possession of cuticular spines upon the surface of the suckers. *C. infundibulum* also possesses cuticular spines on the surface of the scolex. These surficial spines are absent in *C. iola*. *C. infundibulum* may be differentiated from *C. iola* by the number of testes in the mature proglottid, 40–60 for the former, 13–17 for the latter.

DISCUSSION

Guberlet (1916) figured minute cuticular spines on the suckers and scolex for the common chicken tapeworm, *Choanotaenia infundibulum* (Bloch, 1779), but seemingly gave the character little consideration though the family diagnosis denies the presence of spination of the suckers. It appears that *C. infundibulum* and *C. iola* do not adhere strictly to the diagnosis of the family or the genus. It is proposed that the cur-

rent concepts of both the family and the genus as defined by Fuhrmann, 1932, be modified.

The following re-definition of the family *Dilepididae* is proposed:

Scolex more often with an armed rostellum. *Suckers may or may not be armed with cuticular spines.* Genital pores simple or double, unilateral, regularly or irregularly alternating. Uterus more or less sacciform or ramified resolving sometimes into capsules or provided with a parauterine organ—parasites of birds and mammals.

Similarly the definition of the genus *Choanotaenia* must be altered to permit the inclusion of these cognate species.

The following re-definition of the genus *Choanotaenia* is proposed:

Dipylidiinae with rostellum armed with one or two crowns of hooks. *Suckers may or may not be armed with cuticular spines.* Genital pores irregularly alternating. Genital ducts pass between the excretory canals. No seminal vesicle. Testes numerous, situated posterior to or lateral to the female complex. Uterus sacciform or strongly lobed resolving itself into uterine capsules which contain in general only one egg. Parasites of birds.

Table 1.—*Measurements in microns of* Choanotaenia iola

Character	Average	Range
Number of hooks	...	17–20
Length of hooks	32	26–43
Length of rostellum	190	163–219
Width of body of rostellum	65	56–77
Width of crown of rostellum	68	46–97
Length of muscular sac	202	102–239
Width of scolex	356	244–444
Width of suckers	114	92–127
Diameter of cirrus sac	32	26–40
Length of cirrus sac	172	122–224
Diameter of testes	50	43–56
Width of vitellarium	63	46–57
Max. width post. end	557	319–779
Max. width ant. end	424	132–610
Length of recep. sem.	76	56–102
Width of recep. sem.	55	40–66

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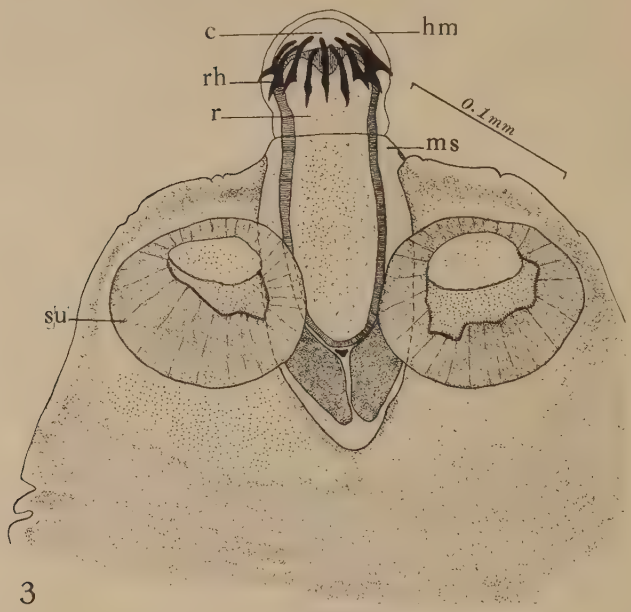
Explanation of Plate, p. 206

Fig. 3. Scolex of *Choanotaenia iola* n. sp.

Fig. 4. Cross-section through sucker.

Abbreviations Used in Plate

c—crown of rostellum
ed—excretory duct
hm—hyalin membrane
n—nucleus
ms—muscular sac
r—rostellum
rh—rostellar hook
sh—sucker spine
sm—musculature of sucker
su—sucker



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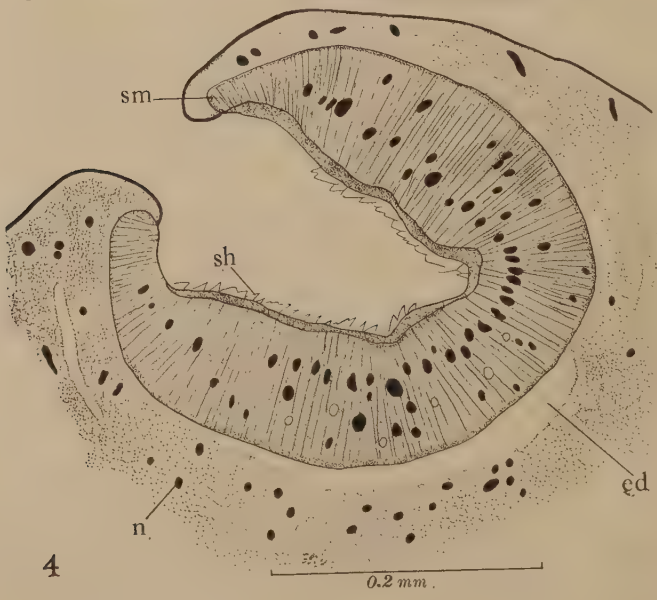

4

LIFE CYCLE OF A FLUKE, *HALIPEGUS ECCENTRICUS* N. SP., FOUND IN THE EARS OF FROGS*

LYELL J. THOMAS

The following life cycle experiments were begun during the summer of 1932 at the University of Michigan Biological Station with the assistance of Arthur Dean Johnson. The earlier work of the writer, Thomas (1934), in infecting copepods with cystophorous cercariae gave a clue as to the method of infection of *Halipegus* sp., a fluke found in the eustachian tubes of frogs in the Douglas Lake Region.

With the publication by Krull (1935) of his account of the life cycle of *Halipegus occidualis* Stafford (1905), the identity of this species of *Halipegus* has become a more complex problem. Although the mature parasites seem nearly to fit the description of Stafford's (1905) *Halipegus*, the cercariae of Krull (1935) differ widely from those of the author and lead to the conclusion that we are dealing with more than one species of *Halipegus*, belonging to the order PROSOSTOMATA, suborder DISTOMATA, superfamily HEMIUROIDEA, family *Halipegidae* Poche, 1925.

In view of the fact that extreme differences exist in the cercariae of these two forms and minor differences in other stages, I propose to call the form herein described as *Halipegus eccentricus* n. sp.

TAXONOMY AND MORPHOLOGY

Halipegus eccentricus n. sp.

ADULT

(Figs. 5 and 14)

Specific diagnosis: Orange-brown worms: body elongate, 6.0–6.5 mm long × 1.8 mm wide as an adult; greatest width just anterior to posterior testis. Cuticula 15 μ thick, sparsely studded with low, glandular tubercles. Oral sucker subterminal, 455 × 434 μ to 621 × 534 μ. No prepharynx; pharynx 192 × 175 μ to 210 × 210 μ. Esophagus extremely short. Ceca sinuous, narrow, rugose, dorsal to other organs, extending nearly to posterior end of body. Acetabulum thick, muscular, 674 × 621 μ to 700 × 674 μ, near middle of body; acetabular opening anterior, eccentric. Testes extracecal, ovoidal, widely separated, oblique; anterior testis 700 × 674 μ to 805 × 796 μ; posterior testis 884 × 796 μ to 892 × 805 μ. Seminal receptacle pyriform, broad, extending dorsoventrally; neck near genital pore, supplied with prostate glands. Ovary nearly median, just anterior to vitellaria, ovoidal, 464 × 437 μ to 490 × 402 μ long, axis at right angles to long axis of body. Oviduct joins Laurer's canal, then common vitelline duct, and continues a short distance to meet oötype with well developed Mehlis' gland. Uterus, packed with eggs, mostly in transverse, S-shaped coils extending from vitellaria to bifurcation of ceca. Genital pore ventral, median, near level of pharynx. Vitelline follicles mostly ovoidal average 280 × 192 μ in diameter, posterior, in two groups, right and left, 4 to 6 or 4 to 5 on a side. Vitelline ducts

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from follicles unite in short common duct in region of ovary. Two lateral ciliated excretory canals, united dorsally near posterior margin of oral sucker, dip towards median line at posterior margin of acetabulum to join thick-walled urinary bladder just anterior to ovary. Bladder median, supplied with villi, discharges through terminal pore. Eggs, straw-colored, mitriform, 67×26 μ to 67×27 μ, broad end operculate; opposite pole, with filament measuring 56–58 μ in length, tapers to fine tenuous recurved tip; fully embryonated when laid. Development in snails *Physa gyrina, P. sayii crassa* Walker, *P. parkeri* Currier, and copepods *Mesocyclops obsoletus* and *Cyclops vulgaris;* cercaria cystophorous, with two streamers in addition to excretory tubes and bulbus; metacercaria in cyclops with everted urinary villi; young flukes in tadpoles.

Type hosts: Rana clamitans, R. pipiens, and *R. catesbiana.*

Location: Eustachian tube of adult frogs; mouth and esophagus of newly metamorphosed frogs; stomach of tadpoles.

Type localities: Black Lake, Fontinalis Run, Burt Lake, and Twin Lakes, Cheboygan County, Michigan.

Type date: July 2, 1933.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 9203.

Less than one per cent of all frogs and snails from Black Lake and Twin Lakes, Michigan, which were examined for *Halipegus eccentricus* were infected. Enough information, however, has been obtained to enable one to show that there are two very closely allied species of *Halipegus* in North America, and perhaps others. Krull (1935) gives measurements of a specimen obtained from *R. clamitans* at Ann Arbor, Michigan, which was 6 mm long and 1.75 mm wide. The oral sucker measured 450 μ in diameter, the pharynx 188×145 μ, the acetabulum 700×720 μ, the ovary 445×310 μ, the anterior testis 445×310 μ, the posterior testis 530×360 μ, the eggs 52×23 μ. There were 4 vitellaria on one side and 5 on the other. I obtained a specimen from *R. clamitans* from Black Lake, Michigan, which measured 6 mm long and 4 mm wide. The oral sucker measured 455×434 μ, the pharynx was 192×175 μ, the acetabulum 674×621 μ, the ovary 464×437 μ, the anterior testis 805×796 μ, the posterior testis 892×805 μ, the egg 67×26 μ, the flagellum-like spine 57 μ long. By the use of Nile blue sulphate, the eggshell and spine were stained a blue color so as to make the attenuated spine sharply visible. There were four vitelline follicles on one side and six on the other. Another specimen taken from the middle ear of *Rana catesbiana,* Twin Lakes, Michigan, measured 6.5 mm long, and 1.8 mm just anterior to the posterior testis. The oral sucker measured 568 μ in diameter, the pharynx 201 μ in diameter, the acetabulum 700×674 μ, the ovary 490×402 μ, the anterior testis 700×674 μ, the posterior testis 884×796 μ, the eggs 67×27 μ, the filamentous spine 58 μ in length. There were four vitelline follicles on one side and five on the other.

The few specimens obtained did not allow for broad comparisons of adult flukes. Krull's measurements for *Halipegus occidualis* based on three of five specimens collected from a large green frog, *R. clamitans,* and relaxed in water showed a range from 6.5 mm in length by 2.0 mm in

width to 5.5 mm in length by 1.5 mm in width. Considering all five the oral sucker varied 380–550 μ in diameter, the acetabulum 590–830 μ in diameter, the pharynx 140–172 μ in width, the anterior testis 490–880 μ in length by 550–710 μ in width and the posterior testis 670–1040 μ in length by 475–670 μ in width. He also notes that there were four vitelline follicles on the right side and six on the left side in one specimen. In another specimen this arrangement was reversed. Two worms had five follicles on the right side and four on the left. In another specimen this was reversed. There were also variations in the 27 eggs measured ranging from 41–55 μ in length by 20–24 μ in width. No measurements of filaments were given except that he states, "The filaments on the eggs appeared to be at least as long as or longer than the egg."

On the basis of measurements alone, one would have a difficult case to prove *H. eccentricus* and *H. occidualis* as two different species. Beaver (1937) has shown that in the same species of a fluke, such as *Echinostoma revolutum*, great variations in size may exist in different hosts. However, adult stages of these two worms definitely support Krull's statement: "It appears probable, therefore, if more than one species of *Halipegus* exists in North American frogs, the specific differences in the flukes will be very minute."

Manter (1938: 34) has proposed the family HEMIUROIDIDAE, subfamily DEROGENINAE, genus *Halipegus*. Early larval stages should be given considerable study before a correct diagnosis may be made for the group. He also considers *Vitellotrema* of Guberlet (1928) and *Genarchella* of Travassos, Artigas, and Pereira (1928) as synonyms of *Halipegus*. *Genarchella* with its lobed vitellaria at the posterior end and *Vitellotrema* with solid masses posterior to and also to the right and left of the ovary appear very much like *Halipegus* in other respects. Variations in the number of vitellaria on either side occur in the same species, therefore the consolidation of these organs into one or partial fusion or separation of them, might be the result of species variation. These conjectures, however, do not solve the problem, but direct our attention to the need for more life history work.

As no complete description of any miracidium of this genus has been available heretofore, the following morphological features are given.

MIRACIDIUM

(Figs, 1, 3, 8, 9, 10 and 12)

Miracidium fully developed when eggs are laid measures 70–82 μ in length; about 30 μ at widest part midway of body. Covered with spiny cuticula; a structureless envelope with eight pen-shaped spines 8 μ long (Fig. 1) crowning anterior end, and alternate rows of lesser spines of diminishing size extending posteriorly (Figs. 9, 10, and 12). Anterior end directed towards operculum of egg. Under spiny cuticular sheath a layer of large squamous epithelial cells (Fig. 3). A large granular, opaque, median gland extending for nearly two-thirds of body length appears to open by pore at anterior end. Five pairs of bilaterally placed ducts and glands (Fig. 8)

somewhat obscure base of median gland. Two large symmetrically-placed glands near anterior end cause ducts on sides to be pushed aside when miracidium contracts. Two flame cells, observed with difficulty, posterior to large median gland. Germ cells numerous, seem to float free in posterior end of body.

Both Creutzburg (1890) and Wagener (1866) saw spine-covered miracidia use the anterior spines to penetrate snail intestines. These large spines may be inverted after entry into the snail and form a depression at the anterior end of the miracidium. Wagener described the miracidia of *Distoma tereticolle,* a fluke from the intestines of the fish *Esox lucius.* He was uncertain whether or not the fine cross-striations below the apical crown of large spines were cuticular folds. Creutzburg records the presence of numerous spines over the body surface of the miracidium of *Distomum ovocaudatum* (=*Halipegus ovicaudatus*). I observed these large spines being inverted and everted, much like the proboscis of an acanthocephalan, when the hatched miracidium was placed in macerated snail intestine.

In an observation made on July 6, 1937, the spine-covered cuticula of the miracidium, which is in one piece, was seen to split longitudinally when the entire specimen was placed in Ringer's solution modified for snails. The larva (Fig. 12) moved back and forth with euglenoid-like movements within this sheath. Finally, (Fig. 13), the larva separated from the sheath, which split into two parts, and assumed in general the appearance of a young sporocyst.

No details of the miracidium are given by Krull; consequently a study of that form would be of great value.

Manter (1926) has described a non-ciliated miracidium of the fluke, *Otodistomum cestoides,* from the skate, *Raia stabuliforis,* in which the bristles or spines occur on five strips or areas radiating from the anterior tip. In the miracidium of *H. eccentricus* there is no evidence of bristle plates. The spine covered cuticula splits into two parts (Fig. 13) when it is shed. The splitting occurs from the anterior tip posteriorly. This is as Creutzburg (1890) observed it in *Dist. ovocaudatum* (=*Halipegus ovocaudatus*). In this respect the miracidium of *Halipegus* differs from the miracidium of *Azygia* and *Otodistomum* and does not agree with Manter's statement (1926: 56) that they have "the very same morphological features." The interpretation of the large median gland by Leuckart (1886) and others as an intestine is questioned by Manter (1926). Its opening directly at the anterior end was observed in *H. eccentricus* to exude a granular substance at the time the spiny cuticula split. This secretion may have a dissolving action on the tunic and host tissues following penetration or during penetration of the snail intestine.

Sporocyst

(Figs. 13, 22 and 23)

Young sporocysts (Figs. 13, 22 and 23) measure 105–169 μ in length and 30–57 μ in width; extremely mobile, appear much like nematodes. Squamous epithelium,

same as under structureless tunic of miracidium, covers body. Sporocysts contracted, show fine transverse striations over surface. Large median gland persistent; not so opaque as in miracidium. Lateral glands and ducts few, atrophying 12 days after eggs fed to snail host. Two flame cells present. Germ balls numerous within body cavity. Posterior end non-granular and extensible. Anterior end (Fig. 23) cups in and protrudes from time to time like a sucker. Three to eight or more cell masses within body cavity interpreted as developing rediae.

As only very young sporocysts were observed, comparisons are of little value, although there seems to be considerable agreement that in both species they are very active and nematode-like.

Glands on either side of this large median gland degenerate to almost insignificance within a week in the young sporocyst whereas the median gland is still persistent (Fig. 23). They may also contribute secretions which are histolytic in character.

Redia

(Figs. 20 and 21)

Mature rediae (Figs. 20 and 21) elongated, ringed with punctate yellow-orange bands, distributed at intervals throughout their length, measure 2.4–2.9 mm (fixed in Gilson's fluid and preserved in 75% alcohol). Alive, some specimens extend to 5.3 mm in length. No birth pore observed. Oral sucker muscular, pyriform, 87 μ in diameter, empties through muscular pharynx directly into sac-like intestine 156 μ in length.

Frequently, rediae are found in the snail host with their walls contracted so that all the cercariae are forced up to the anterior end which bulges with their presence. This may be a part of the elimination process by which they come to the outside.

The rediae of *H. eccentricus* are a little larger than those of *H. occidualis*, and Krull (1935) does not mention the distinct series of orange-yellow bands which are present in my specimens.

Cercaria

(Figs. 15, 16, 17, 24 and 25)

Cercaria encysted within tail or bulbus which measures 87 × 174 μ in diameter; outer wall about 1 μ thick. Bulbus with fluted excretory appendage 4 μ long bearing six ridge-like alae; to the side near base of excretory appendage a pyriform organ with waste granules. A tube normally coiled within bulbus may be inverted or everted at a point directly opposite to fluted excretory appendage; measures about four times the diameter of bulbus in length; terminal ending bulb-like, followed for a short distance by region thickly studded with refractive, punctate cells. Two ribbon-like epithelial streamers of varying length attached on either side at base of excretory appendage.

Cercaria body about 140 μ long, opaque, cylindrical, coiled nearly in figure 8 within bulbus. Body ejected approximately 200 × 50 μ; surface covered with fine, denticulate, cuticular ridges. Oral sucker 20 μ in diameter; acetabulum, a short distance posterior to pharynx, 21 μ in diameter. Pharynx 15 μ in diameter. No preesophagus; very short post esophagus. Intestinal ceca lateral, extending nearly to thick-walled excretory bladder. Only trace of inverted U-shaped excretory ducts visible between acetabulum and pharynx. No flame cells visible.

The cercariae of *H. occidualis* eject the excretory tube (delivery tube of Krull) from the bulbus or cyst at a point near the handle, although

in his description he says, "The delivery tube, considered as an appendage of the highly specialized tail, is differentiated at about the same time that the constriction between body and tail appears. In the subsequent development of the cercaria the handle is differentiated from the sacculate portion of the tail. The opening of the sacculate part during development is on the side opposite the handle, and from this opening project the developing delivery tube and cercaria body." This is as I observed it in *H. eccentricus* (Fig. 19), but with this difference, that at a point directly opposite the handle, both cercaria and excretory tube are ejected from the bulbus. Furthermore, in the cercaria of *H. eccentricus* two long epithelial streamers grow out from the bulbus, one on either side of the base of the handle (Figs. 15, 16, 17, 24, and 25). No such streamers are mentioned or figured for the cercaria of *H. occidualis* (Krull, 1935: 131–134; plate 4, Figs. 9 and 12). The esophagus of the cercaria of *H. occidualis* is long and narrow but in *H. eccentricus* it is so short that the ceca seem to unite almost directly to the pharynx.

Both Krull (1935: 131) and Willey (1930: 486) describe the ejection of cystophorous cercariae through the excretory tube which Krull has called the delivery tube. Throughout my observations upon the cercariae of *Halipegus eccentricus,* I have never seen them ejected through this tube. Both cercaria and tube come out of the bulbus at a point opposite to the excretory appendage. Both may be out at the same time or only one and not the other (Figs. 15, 16, 17, 24, and 25). I have observed the excretory tube, everted at the time the cercaria was pounced upon by a cyclops, to have a jointed or telescoped appearance much like that shown by Willey (1930) for *Cercaria projecta* and that by Rothschild (1938) for *Cercaria sinitzini.* I have followed the terminology of Cort and Nichols (1920) as they have described a cystophorous cercaria, *Cercaria californiensis,* from the snail, *Helisoma occidentalis,* which is similar in many respects to that of *H. eccentricus.*

Metacercaria

(Figs. 7 and 18)

Metacercariae, fully extended in Ringer's solution modified for Crustacea, measure 612–744 μ in length; anterior end to acetabulum about 437 μ in length; acetabulum to posterior end about 175 μ in length. Oral sucker 61 μ in diameter. Pharynx 26 μ in diameter. Acetabulum about 131 μ in width with eccentric opening; muscles more developed posteriorly than anteriorly. Two lateral, orange-colored, rugose ceca extend nearly to posterior end of worm. Inverted U-shaped excretory ducts, filled with dark refractive excretory granules, extending from base of oral sucker lateral to ceca, to join in median line, posterior to acetabulum, a heavy-walled urinary bladder with numerous villi. Bladder opens by terminal pore. In coelom of cyclops, bladder villi everted. Cuticula 2 μ thick sparsely studded with small papillose tubercules, also covered with small transverse, denticulate ridges.

Rothschild (1938: 44–45) has described the development of a cercaria in which the large epithelial tubercules are the remains of large nuclei.

The only feature of the adult that is also consistently evident in the metacercarial stages of *H. eccentricus* is the opening of the acetabulum. This heavy muscular organ with sphincter muscles more developed posteriorly than anteriorly, causes the opening to be eccentrically placed, whereas in Krull's specimens of *H. occidualis* examined by the writer, the opening of the acetabulum is exactly centered, due to uniform distribution of muscles around it. The same variations in vitellaria occur in both species. Similar variations exist in testes and ovaries.

The larval stages, on the other hand, show sufficiently wide variations to justify the establishing of another species.

In Krull's description of a metacercaria taken from a naturally infected dragonfly, *Libellula incesta* (1935: 124), the body measured 478 μ in length and 182 μ in width. This is smaller than those measured by the writer which had been in *Mesocyclops obsoletus* 19–30 days. The largest was 744 μ in length and not advanced enough to show formed ovary and testes as Krull (1935: plate 4, Fig. 14) figures for *H. occidualis*. In his specimen there is definite development of the primordia of Mehlis' gland and the uterus. The acetabulum in his metacercaria measured 154 × 170 μ; in my specimens it is only about 131 μ in diameter. The adult acetabulum of *H. occidualis* measures 714 μ in diameter and opens uniformly; in my specimens it ranges from 674 × 621 μ to 700 × 674 μ, opens eccentrically and is slightly smaller. Krull (1935) does not mention the papillose tubercules and fine denticulate transverse ridges which are plainly evident in the metacercaria of *H. eccentricus*.

EXPERIMENTS

In 1934 I advanced the hypothesis that copepods act as intermediate hosts in transferring the larval stages of hemiurids to second intermediate hosts such as dragonflies, *Sagitta*, medusa, etc. This Krull (1935) assumes to be valid in the case of *Halipegus* infecting dragonflies. The experimental evidence, I find, does not support such a view in this case, and the salient points in this regard are given abstract form, Thomas and Johnson (1934). Since then, additional evidence and information have been added. The following materials were given in part by Thomas (1937) in abstract form before the American Society of Parasitologists.

Infecting the Copepod Host

A heavy infection of cystophorous cercariae found on July 2, 1933, in a single snail, *Physa gyrina*, was used for feeding experiments until no more cercariae were given off on July 8. The snail was placed in a large evaporating dish in shallow water to which were added large numbers of *Cyclops vulgaris* and *Mesocyclops obsoletus*. Daily examinations were made of the copepods. From 40 to 50 per cent became infected

with one to three active metacercariae. Infective stages were reached in 14 to 21 days in the body cavity of *Cyclops vulgaris* and from 19 to 30 days in *Mesocyclops obsoletus*. Those from *Cyclops vulgaris* attained a size of 612×230 μ.

Observations were made in the summer of 1937 to determine how cyclops fed upon cysts of *Halipegus*. While the cercariae lie quiescent for the most part at the bottom of the dish, occasional individuals were noticed which repeatedly thrust the excretory tube out to its full length and then hauled it in again. The copepods pounced upon them; the excretory tubule shot out, but away from the mouth of the copepod. Within a few seconds after the feeding, the cyclops were observed by means of a compound microscope. Each cercaria was seen working its way back into the intestine by a series of hitching movements. The denticulate ridges of the cuticula seem to serve as holdfasts following each forward thrust of the parasite's body. Possibly glandular secretions of the parasite together with its cuticular ridges and hitching movements bring about the cytolysis of host cells and thus open the way into the coelom. Within this cavity the specimen became very active and within six hours after entry into the body cavity, orange oil-like globules which had been present in the copepod were to be seen in the intestinal lumen of the fluke.

The delivery of cercariae into the copepod, as described for *Halipegus occidualis*, does not occur in *H. eccentricus*. The normal feeding reaction of copepods, whether it be upon protozoa, eggs, coracidia, or larval nematodes, is to stop briefly after feeding as though stunned. At such a time one can push them over with a fine needle. A few seconds later they dart away upon being disturbed. I observed no paralyzing effect on the copepods upon eating the cercariae as suggested by Krull (1935: 139). It was not unusual for a large cyclops to gorge its intestine with cercariae but only a few reached the body cavity within the course of a day. This same phenomenon I have observed in copepods feeding upon the larvae of the nematode *Camallanus trispinosus*. The intestine would be gorged following the introduction of the larvae into the water with the copepods yet by the next day only three or four larvae would be in the body cavity, and an examination of the copepod fecal pellets showed the others to have been digested. This same thing was observed in the case of *Halipegus* cercariae.

Infecting the Snail Host

Six young *Physa sayii crassa* Walker were placed in an evaporating dish containing the eggs of *Halipegus eccentricus* July 14, 1933. The eggs were covered with lake water to a depth of about 5 mm. Some days later, July 28, one was examined and found to have several sporocysts (Fig. 23). It was also noticed that the fecal pellets of the snails contained

many unhatched eggs as well as empty shells. The miracidia (Fig. 9) in these eggs which had passed through the snail intestine seemed to be alive and normal. The sporocysts measured 105–165 μ by about 24 μ and were very active. A dozen *Physa sayii crassa* served as controls.

Again on August 14 another snail from this same lot was examined and found to contain a dozen fully developed rediae each bearing fifty or more cystophorous cercariae. The rediae measured 805 × 200 μ. The cercariae were identical with the cystophorous cercariae taken from *Physa gyrina* in a natural infection July 2, 1933. The bulbus of these experimentally reared cercariae averaged 76 μ in diameter. It was evident that it took about a month for the cercariae to develop following the penetration of the miracidia into the snail.

Two adult *Halipegus eccentricus* were collected from the eustachian tube of an adult bull frog, *Rana catesbiana,* taken July 5, 1937, at Twin Lakes, Cheboygan County, Michigan. By holding the frog with open mouth up to direct sunlight the worms could be seen through the tympanic membrane as moving orange-brown objects. This proved to be a quick and accurate method for determining infections in other species such as *R. clamitans* and *R. pipiens.* These worms were placed in Ringer's solution modified for amphibia in a two-inch stender dish. Here they gave off an abundance of embryonated eggs and others not so well developed. Five young snails, *Helisoma trivolvis,* from an uninfected lot obtained from Ann Arbor, Michigan, were added to the dish and then observed at 5:00 PM, July 6, 1937. All eggs had been eaten and only empty shells and undeveloped eggs remained in the fecal pellets. On August 6, 1937, six more *H. trivolvis* snails were placed with eggs. Examination of all the snail hosts on August 15, 1937, showed only two infections. Snail hosts were identified by Frank C. Baker, Museum of Natural History, University of Illinois, to whom grateful acknowledgment is given.

Attempts to Infect Dragonfly and Damselfly Nymphs

It was known that Sinitzin (1905) had observed *Halipegus ovicaudatus* metacercariae develop in the body cavity of *Colopteryx virgo.* Also, Krull (1935) obtained a metacercarie from a naturally infected dragonfly, *Libellula incesta* Hagen, taken at Beltsville, Maryland, and reports a metacercaria similar to that of *H. occidualis* collected from the damselfly, *Lestes rectangularis* Say, taken in the Douglas Lake Region of Michigan. With these facts in mind, the feeding of infected cyclops to various dragonfly and damselfly nymphs was attempted.

Although the infected copepods had carried their infection in some cases from one week to about thirty days, no infections were obtained when they were eaten by the nymphs. In each case, the feces of the nymphs showed orange fat bodies and parts of copepods but no metacer-

cariae in the body cavity when dissected. Newly hatched nymphs of dragonflies were exposed directly to the cercariae of *H. eccentricus* without results.

It seems probable that the maturing or mature metacercariae will not transfer to a second intermediate host. On the other hand, specific dragonfly or damselfly nymphs may be required if the cercariae are to develop directly in such hosts.

Field Observations

A natural heavy infection of cystophorous cercariae was found in *Physa gyrina* on July 2, 1933, from Black Lake, Michigan, together with a rather light infection of stylet cercariae. As the cystophorous cercariae sink to the bottom of the container, some may have passed unnoticed in the routine examination for more active forms.

The examination of *Rana pipiens* and other frogs from this region was continued throughout the summer. On August 2, 1933, a young, newly metamorphosed *Rana pipiens* was found with one immature *Halipegus* in the stomach and two slightly larger ones in the mouth. Again on August 9, 1933, one *Rana clamitans* and one *R. pipiens*, newly metamorphosed, had three and four immature *Halipegus* respectively, in the esophagus.

A large *Rana pipiens* from Black Lake examined July 14, 1933, had four adult worms, two in each eustachian tube. These worms were sexually mature and their eggs were used for feeding experiments and for the study of the miracidia. A large *R. clamitans* from this same region was also found with one adult *Halipegus* in the eustachian tube. Two adult specimens of this same fluke, *H. eccentricus*, were obtained in the summer of 1937 and one specimen in the summer of 1938 from the eustachian tubes of *Rana catesbiana*, Twin Lakes, Cheboygan County, Michigan. Another adult frog, *R. clamitans*, from Fontinalis Run, Burt Lake, Michigan, had two mature specimens, one in each eustachian tube.

I wish to express my appreciation for the help received from Professors W. W. Cort, George R. LaRue, Donald B. McMullen and others in securing some of the materials for this study.

Infecting the Final Host

Six laboratory reared *Rana pipiens* tadpoles were placed in shallow water in finger bowls with infected copepods, July 28, 1933. One tadpole was examined August 1, 1933, and an immature fluke was taken from the cardiac end of the stomach. It was noticed that the intestinal ceca of the fluke, which were brightly colored with orange fat droplets while in the coelom of the copepod, were now clear. When observed on a tilted slide the young fluke always climbed up the inclined plane even

though tilted at a 45° angle. This was repeated many times with the same result. Perhaps this negative geotropism is partly responsible for the migration of the fluke into the mouth of the frog during metamorphosis. Differences in pH may also be contributing factors. This one specimen measured 761 μ in length; the distance from the acetabulum to the anterior end was about 455 μ and the distance from the acetabulum to the posterior end was about 175 μ; the diameter of the acetabulum was about 131 μ. The villi of the urinary bladder which were everted while in the cyclops were now inverted within the bladder. They seem to be a continuation of the external epithelium.

The other five tadpoles were examined on August 15, 1933. Two had three each, two none and the other one in the cardiac end of the stomach. This experiment was repeated again the summer of 1937, but only one infection was obtained.

In brief, the writer has demonstrated from the morphological study of *Halipegus eccentricus* n. sp. in the earlier part of this paper that it is scarcely distinguishable from *H. occidualis*. However, my subsequent studies on the life cycle stages of *H. eccentricus* clearly show that these two flukes are distinct species.

SUMMARY

From morphological studies, field observations, and laboratory experiments the life cycle of *H. eccentricus* may be summarized as follows:

1. Cystophorous cercariae are swallowed by copepods in which they undergo development, free in the coelom, to the infective stage within two to three weeks.
2. The infected cyclops are swallowed by tadpoles.
3. The worms remain, with little development, in the cardiac end of the stomach until beginning metamorphosis into a frog when migration up to the esophagus begins.
4. In the mature frog the parasites lodge in the eustachian tubes.
5. Embryonated eggs passed in the feces of the frog hatch when eaten by the snails *Physa sayii crassa* Walker, *P. parkeri* Currier, *P. gyrina,* and *Helisoma trivolvis*.
6. The non-ciliated miracidium penetrates the snail intestine by means of its spiny tunic and sheds it to become the sporocyst.
7. The sporocyst continues to grow and develops three to eight or more rediae.
8. Within about one month after infecting the snail host, the orange-banded rediae each produce fifty or more cystophorous cercariae.

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Explanation of Plates

All drawings, unless otherwise indicated, are made by the use of the camera lucida. The projected scales have the following values: Figs. 1, 8, 10, 12, 13, and 22 = 0.01 mm; Figs. 4, 6, 7, 9, 15, 16, 17, 18, 21, 23, 24, and 25 = 0.5 mm.

Plate I

Fig. 1. Anterior end of miracidium showing crown of pen-like spines.
Fig. 2. Freehand sketch of anterior end of miracidium showing action of spines in penetrating snail intestine.
Fig. 3. Freehand drawing of body layers at different levels to show arrangement in living miracidium.
Fig. 4. Young *Halipegus eccentricus* from cardiac stomach of metamorphosing *Rana pipiens*, August 10, 1937.
Fig. 5. *Halipegus eccentricus* adult from eustachian tube of *Rana clamitans*.
Fig. 6. Dorsal view of cyclops, fifteen minutes after ingesting three *Halipegus* cercariae, July 8, 1937.
Fig. 7. Living metacercaria in Ringer's solution for crustacea: in laboratory reared *Cyclops viridis* one week, July 10, 1933; ceca with orange oil-like globules; bladder villi everted.
Fig. 8. Inner arrangement of glands; flame cells; germ cells of miracidium in operculate egg.
Fig. 9. Fully embryonated egg from frog feces.
Fig. 10. Hatching of the miracidium in intestinal juice of snail.
Fig. 11. Drawing of Krull's specimen of adult *Halipegus occidualis* Stafford (1905) for comparison with *H. eccentricus*.
Fig. 12. Miracidium in Ringer's solution for snails undergoing euglenoid-like movements within spiny tunic.
Fig. 13. Splitting of the spiny tunic after penetrating snail intestine to form a sporocyst.
Fig. 14. Young *H. eccentricus* from esophagus of newly metamorphosed *R. clamitans*.

Plate II

Fig. 15. Bulbus of cystophorous cercaria with cercaria excysted; excretory tube within bulbus; taken from dish shortly after exposing cercaria to cyclops.
Fig. 16. Cercaria excysted and attached; excretory tube within bulbus.
Fig. 17. Cystophorous cercaria shortly after escape from snail host.
Fig. 18. Metacercaria in body cavity of *Mesocyclops obsoletus* (= *C. leuckarti* Claus) infected July 15, 1937, showing bladder villi everted.
Fig. 19. Formation of excretory tube and bulbus of developing cercaria from experimental infection.
Fig. 20. Orange-banded redia with cercaria; experimental infection.
Fig. 21. Young redia taken from natural infection.
Fig. 22. Sporocyst with developing rediae from experimental infection.
Fig. 23. Young sporocyst showing cupped sucker-like anterior end and germ balls.
Fig. 24. Bulbus with excretory tube extended after the eating of the cercaria by cyclops.
Fig. 25. Cercaria excysted and excretory tube everted.

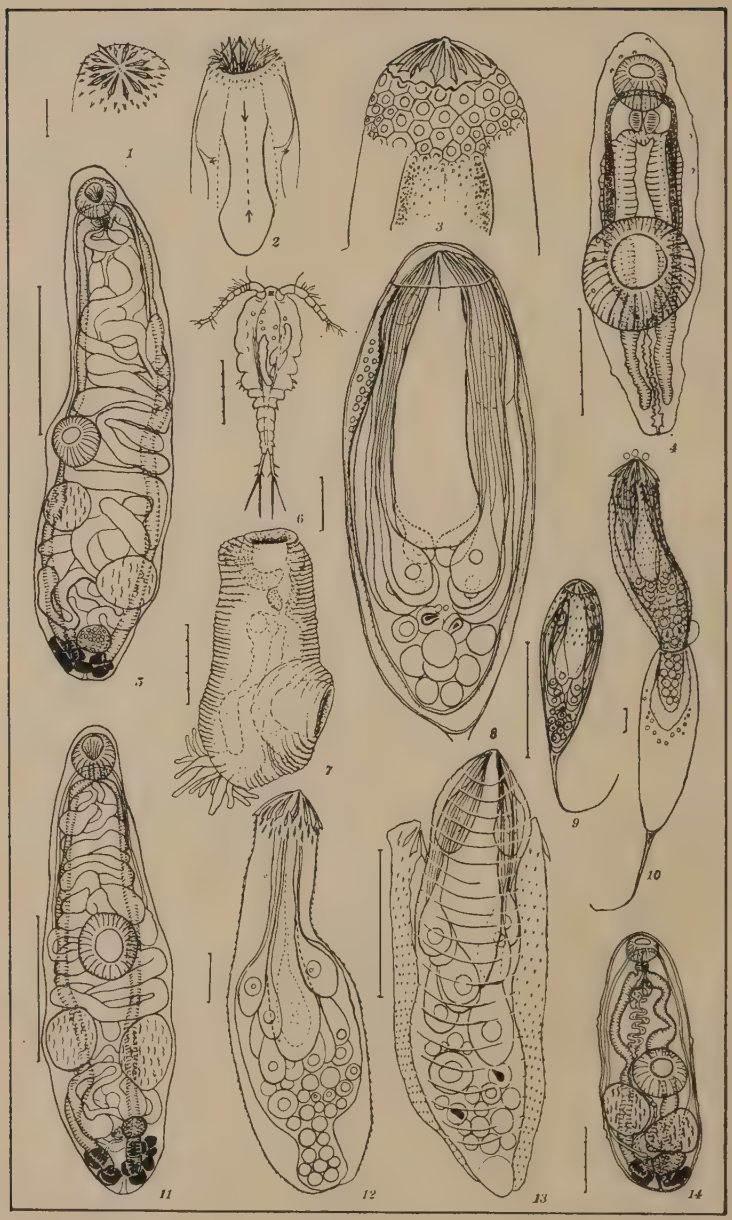

PLATE I

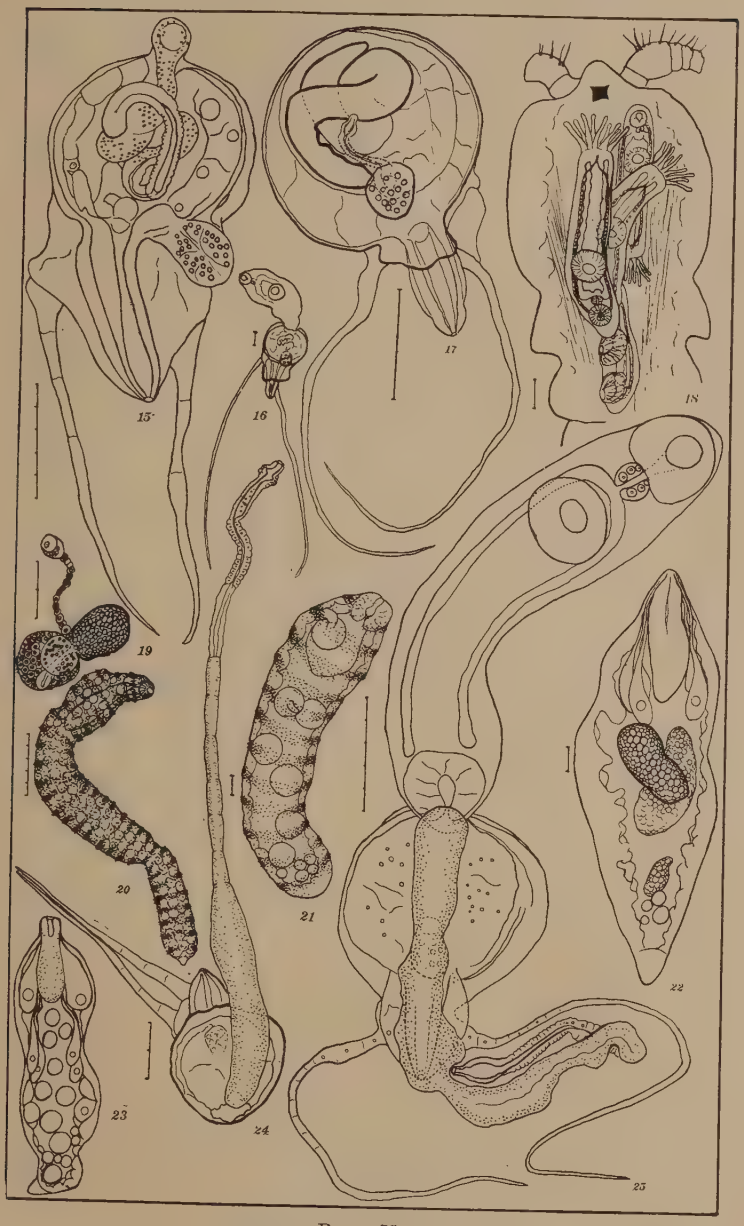

PLATE II

STUDIES ON THE PRE-CERCARIAL DEVELOPMENT OF *STICHORCHIS SUBTRIQUETRUS* (TREMATODA: PARAMPHISTOMIDAE)*

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Thirty-two *Stichorchis subtriquetrus* (Rudolphi) Näsmark, 1937, a paramphistomid trematode, were recovered on March 29, 1938, from the intestinal cecum of a beaver, *Castor canadensis carolinensis,* killed on the same day at Gonzales, Louisiana. Advantage was taken of the material to study the pre-cercarial development of this form, in which there is the exceptional condition of a single fully formed redia produced within the miracidium before the egg hatches.

The adult worms were removed from the walls of the cecum and placed in a fingerbowl filled with tap water, where they were allowed to lay their eggs. After several hours they had ceased actively contracting and were measured without stimulation and without pressure by means of calipers. The smallest adult measured 6.5×4.0 mm, the largest 15.0×6.5 mm, the average being 10.2×5.2 mm.

THE EGG AND DEVELOPING MIRACIDIUM

Several thousand eggs were set aside and allowed to hatch. Twenty-five eggs were measured, the smallest being 118×82 μ, the largest 154×118 μ, and the average 132×96 μ. The opercular openings of five eggs averaged 29 μ, with the edges of both the eggs and the operculum slightly scalloped, each scallop being about 1 μ long. The ovum is in the center of the vitelline mass, which consists of a large number of translucent, brownish cells. The opacity of the surrounding vitelline cells of the egg made accurate observation of the ovum and early embryo difficult. The embryo at the end of twelve hours is still obscured to such an extent that no clearly defined outline can be seen. However, at the end of this time it is approximately 15 μ in diameter. By the end of the first week the embryo is sufficiently large to measure with more accuracy. It appears as a spherical, semi-translucent mass near the center of the vitelline cells and measures approximately 40 μ in diameter. During the second week of development there is a slow but steady increase in size with a concomitant loss of the spherical shape. At the end of this time the smallest embryo observed measured 42×46 μ; the largest measured 49×108 μ.

During the third week several of the more characteristic features of the miracidium became evident. The first of these to be observed was

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the flame cells, which were first seen in a 15-day-old embryo which had reached a size of 49×92 μ. Forms of this size show a clearly formed germ mass in the posterior half of the body, which is destined to give rise to a single redia (Fig. 1). The germ mass in miracidia of the above size fills the posterior third of the body but shows none of the morphological features which appear in subsequent development. In miracidia which measured approximately 102×49 μ there is a slight development of the so-called primitive gut. It is at this stage that the miracidium first begins to move. Movements are frequent and strong, consisting of slow contractions at first one point and then another. In slightly larger forms, 108×49 μ, the cilia, arranged in transverse rows on the body, are first seen.

During the development the vitelline cells are gradually broken down. Their contents seem to coalesce, resulting in the formation of large globules which are apparently of an oily nature.

The miracidium continues to increase in size until it extends from the opercular cap to the posterior end of the shell (Fig. 2). In some instances it may be slightly longer than the egg, and in such cases it is forced to bend upon itself. Miracidia reach this size at about the end of the third week of development and are ready to hatch. Hatching efforts apparently consist of strong contractions of the body which force the apical papilla against the operculum. This muscular contraction is accompanied by violent beating of the cilia. Hatching in *S. subtriquetrus* thus seems to be due entirely to muscular and ciliary activity on the part of the miracidium. About 82 per cent of the eggs hatched.

THE MIRACIDIUM

The miracidium is elongate and covered with rows of cilia, except for the apical papilla (Fig. 3). Fifteen living miracidia had the following measurements: minimum length 151 μ, maximum length 224 μ, average length 208 μ, minimum width 47 μ, maximum width 90 μ, and average width 72 μ. Freshly hatched miracidia are active for eight or ten hours, after which they become sluggish and come to rest on the bottom of the dish until death and disintegration occur. The cilia, which are distributed in transverse rows on epidermal cells of the pattern 6:8:4:2, are 4 μ long on tier one, 8 μ long on tiers two and three, and 11 μ long on tier four (Fig. 4). In a miracidium which measures 183×64 μ the lengths of the cells are from the anterior end 36, 61, 54, and 32 μ. The nuclei of the epidermal cells were studied by suspending living miracidia stained with methylene blue or neutral red in a hanging drop. The nuclei are rodlike or slightly curved and stain deeply. They are located near the posterior ends of the cells in tiers one, two and three, but are near the anterior ends of the cells in tier four.

The primitive gut of the miracidium is a large sac, filled with a granular substance, opening on the apical papilla. Its shape varies according to the state of contraction of the miracidium. No penetration glands such as those which have been described for other amphistome miracidia could be seen.

There are two large flame cells 10×6 μ lateral and slightly anterior to the middle of the body of the miracidium (Fig. 5). From each of these cells a duct 4 μ in diameter leads posteriorly to the end of the body, then turns back on itself for a distance, making a final posterior turn slightly posterior to the flame cell, and opens to the exterior between epidermal cells of tiers three and four.

The posterior two-thirds of the body of the miracidium is filled by a single, well-formed redia. There are no other germ balls in the miracidium and only one redia is formed in each. Twenty living rediae in miracidia under slight pressure had the following measurements: minimum length 58 μ, maximum length 162 μ, average length 126 μ, minimum width 39 μ, maximum width 97 μ, and average width 63 μ. The redia is capable of contraction and extension independent of the miracidium. A muscular pharynx about 24×27 μ is present in the anterior end, from which a short esophagus extends posteriorly ending in an imperforate plug 3 μ thick which separates it from the gut (Fig. 6). The rhabdocoele gut is about 27×19 μ with walls 3 μ thick, leaving a lumen about 22×10 μ. The posterior half of the redia and sometimes even the region around the pharynx contains germ balls about 4 μ in diameter. There are two pairs of flame cells in the young redia, the anterior pair lateral and slightly anterior to the middle of the body and the posterior pair lateral to the posterior end of the gut. From each flame cell a tubule runs toward common ducts on each side which open to the exterior of the redia at a point slightly behind the middle of the body. The flame cells of the redia are about 6×3 μ.

The redia possesses two conspicuous sac-like structures filled with a material that is similar in appearance to the content of the miracidial gut. In every instance one of these structures is much larger than the other. The larger is approximately twice the length and width of the smaller. They empty through individual ducts which open on both sides of the body, lateral to the pharynx. Subsequent observations made on rediae indicate that these bodies may be penetration glands.

The redia remains in the posterior half of the miracidium until ready to be liberated. Many observations were made on the process of liberation from the free-living miracidium and it always occurs in the same fashion. It is accomplished primarily through the activity of the miracidium, but there is a possibility that the content of the "penetration glands" of the redia may have some histolytic action which aids in the

destruction of the miracidial tissues. The process of liberation is initiated by strong contractions of the circular muscles of the miracidium at the posterior end of the body. Such action forces the redia tightly into the anterior half of the body. Within a few minutes the anterior end ruptures and the redia is set free into the surrounding water. The process of liberation in the tissues of the snail was not observed, but subsequent findings indicate that the process is probably the same.

The intermediate snail host was experimentally determined by exposing snails to the free-living miracidia on April 23, 1938. The amphibious snail *Fossaria parva* was the only one of many species tried which could be infected. The miracidia of *S. subtriquetrus* reacted toward the snail hosts much as do those of *Cotylophoron cotylophorum* as described by Bennett (1936). They are not strongly attracted and were not seen penetrating the snail. However, after exposure the snails were fixed, sectioned and stained, and in this way it was determined that the miracidia penetrate the mantle (Fig. 7). Apparently there is no immediate significant structural change in the miracidium as a result of penetration, since it retains its epidermal cells.

The exact time at which the redia is liberated in the snail was not determined. However, in snails fixed and sectioned 48 hours after infection free rediae were found in the mantle (Fig. 7). The figure shows clearly a redia lying in close proximity to an empty miracidium. There can be little doubt that the redia was recently liberated from it because the nuclei of the epidermal cells of the miracidium were still clearly evident. There was no sporocyst stage observed. According to Wesenberg-Lund (1934) omission of the sporocyst stage in a trematode life history has been reported before by Szidat (1932) in the monostome *Trachecophilus sisowi* Skrj., which is synonymous with *Typhlocoelum cymbium* (Diesing, 1850) Kossack, 1911 (Family CYCLOCOELIDAE). In this worm the redia develops in the miracidium. There is only one redial generation and the cercariae encyst in the snail until eaten by the final host. Stunkard (1934) worked out the complete life history of *Typhlocoelum cymbium* and confirmed the account of Szidat.

A number of snails were fixed at intervals but so many proved to be uninfected that growth data at regular intervals were not secured. Following observations made after a two-day interval no other rediae were seen until the 17th day. One large redia was found in the liver of a snail dissected on May 9, 1938. The "penetration glands" of the mother redia were gone and it is believed that they were exhausted during its migration from the mantle. It measured 0.62 × 0.36 mm and contained fifteen daughter rediae at varying stages of development. Only three of the daughter rediae were sufficiently developed to show movement. The pharynx, esophagus, gut, flame cells, "penetration glands," and germ balls were present in them also.

On May 13, 1938, 21 days after exposure, another snail was dissected and one large redia was found, which measured 0.64 × 0.16 mm and contained a large number of developing rediae. In addition to the single mother redia six daughter rediae were found in the tissues of the snail. The largest of these measured 0.20 × 0.09 mm. Other snails were dissected on the 24th and 27th days after exposure to the miracidia but the mother rediae found showed no increase in size or other morphological change. By the 27th day the daughter rediae had increased to a size of 0.29 × 0.10 mm. All but a very few of the infected snails had been dissected or had died by May 20, 1938, and the remainder died shortly afterward. For this reason no additional stages were obtained.

THE MOTHER REDIA

The following description of the mother redia is based on a very few living specimens taken from snails at irregular intervals (Fig. 8). The largest specimen was dissected from its host 21 days after infection. It measured 0.64 × 0.28 mm. On the 22nd day the largest measured 0.47 × 0.10 mm, and on the 27th day the largest specimen measured 0.56 × 0.27 mm. The mature rediae are active for a considerable period of time in physiological salt solution. This activity consists of the usual alternate contraction and relaxation of the circular and longitudinal muscles.

The contraction of the longitudinal muscles produces a conspicuous backward fold of the body wall, which completely encircles the middle of the body. The largest daughter rediae are found in the region of the fold tightly pressed against the posterior portion of the gut. The fold may project backward for a distance of 72 μ and laterally for 36 μ.

The only parts of the digestive apparatus studied were the pharynx, the esophagus, and the rhabdocoele gut. The largest redia seen possessed a pharynx which measured 36 μ in length by 43 μ in width. The esophagus measured 54 μ in length and the gut 0.210 × 0.054 mm. The shape and measurements of the latter structure vary considerably with the contraction of the redia. The "esophageal plug" of the immature redia mentioned above was not observed in any older forms, but it must disappear very soon after the redia is liberated from the miracidium.

The redia described here contained seven fairly well-developed daughter rediae and ten not nearly so advanced. This is the largest number of daughter rediae found in any one mother redia. The most fully developed daughter redia measured 144 × 72 μ. The birth pore of the mother redia is located on the ventral surface at about one-tenth the total length from the anterior end of the body. It is a prominent structure which projects for a distance of from 10 to 15 μ (Fig. 8).

The excretory system is very similar to that described for other amphistome rediae. It consists of three flame cells, their ducts, a small

bladder and an excretory pore on both sides of the body. The anterior flame cells are located approximately one-tenth of the body length from the anterior end; the midde pair is usually found slightly posterior to the middle of the body; the third pair is about one-sixth of the body length from the posterior end. The redia while in the miracidium has only two pairs of flame cells, and the time at which the third or middle pair of cells develops was not determined. The duct from the middle cell joins that of the anterior one near its termination and the posterior flame cell duct empties into the bladder in common with the anterior duct. The excretory bladder is approximately 16 μ long and opens to the exterior by a short duct near the center of the body. The excretory pores are on the lateral margins of the body.

A mass of tissue lying above the esophagus, measuring about 35 μ in diameter, is probably the "brain mass."

THE DAUGHTER REDIA

The germ balls which are to become daughter rediae are present in the mother redia before it is liberated from the miracidium. The largest of these measured 5 μ. No other observations were made on daughter rediae prior to the 17th day after infection of the snail host. A single mother redia was dissected from its host on May 9, 1938; it measured 0.62×0.36 mm and contained fifteen developing rediae, only two of which were active. These two were approximately equal in size, the slightly larger one measuring 0.18×0.08 mm. The next in size was considerably smaller, measuring only 0.12×0.10 mm. However, all three possessed the pharynx, gut, "penetration glands," flame cells, and germ balls. The remaining daughter rediae formed a series decreasing in size toward the posterior end of the mother redia.

In the most fully developed daughter redia the pharynx measured 29×25 μ and the gut was 65×36 μ. The "penetration glands" are unequal in size as they are in the immature mother redia. The larger measured 47×36 μ, while the smaller was only about half as large. These "glands" lie on each side of the body and have individual ducts which open laterally at the level of the pharynx. The largest germ ball, of which there were several, measured 54×36 μ. The excretory system is similar to that of the mother redia, consisting of three pairs of flame cells and their ducts. The system is developed prior to the birth of the daughter rediae.

The first daughter rediae to be found free in the tissue of the snail were obtained on the 21st day after infection. Only two were seen, the larger measuring 0.21×0.10 mm, and the smaller 0.20×0.09 mm. There were no significant morphological changes that could be observed other than the disappearance of the "penetration glands." The function of

these "glands" is obscure and it is surprising to find that they disappear so obviously early after the birth of the daughter redia. An obvious assumption we can make is that they are exhausted by the daughter redia during its early migration. If this assumption is true then the "glands" presumably have some histolytic action which aids the redia in becoming established in a suitable location in the snail host.

The similarity of this amphistome life history to that of the monostome *Typhlocoelum cymbium* points to a possible phylogenetic relationship between members of these two groups.

SUMMARY

Stichorchis subtriquetrus (Rudolphi) Näsmark, 1937, is reported from the beaver, *Castor canadensis carolinensis,* in Louisiana. The development of the miracidium is exceptional in that a single fully formed redia develops prior to the hatching of the egg. The eggs begin hatching at the end of the third week and continue for several days. The redia is unusual in that it possesses a pair of structures which are called "penetration glands."

The amphibious snail *Fossaria parva* serves as an experimental intermediate host. The miracidium has a ciliated epidermal cell formula of 6:8:4:2 and retains these cells during and for at least 48 hours after penetration. The miracidium does not develop into a sporocyst but disintegrates upon the liberation of the redia.

The redia migrates to the liver of the snail, apparently using the contents of its "penetration glands" in the process. Daughter rediae are born approximately twenty-one days after infection of the snail. They possess "penetration glands," similar to those of the mother redia, which are exhausted shortly after birth.

The results of this study suggest a possible phylogenetic relationship between some members of the amphistome and monostome groups.

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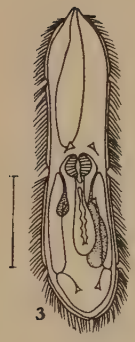
Explanation of Plate

All figures were drawn from living material, except Fig. 7, and all with the aid of a camera lucida. Scale for Figs. 1–6 inc. and Fig. 8 = 0.05 mm; for Fig. 7 = 0.025 mm.

Fig. 1. Developing miracidium showing germ mass and flame cells.
Fig. 2. Mature miracidium prior to hatching.
Fig. 3. Miracidium immediately after hatching.
Fig. 4. Miracidium showing the arrangement of the epidermal cells.
Fig. 5. Miracidium showing the flame cells and the course of their ducts.
Fig. 6. Redia as it appears in the mature miracidium.
Fig. 7. The redia immediately after emerging from the miracidium in the mantle of the snail.
Fig. 8. Mature mother redia.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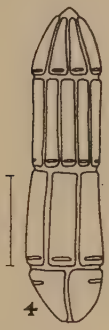
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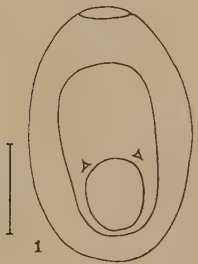
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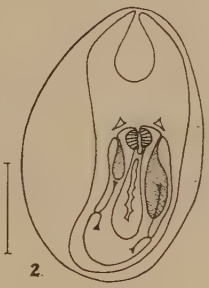
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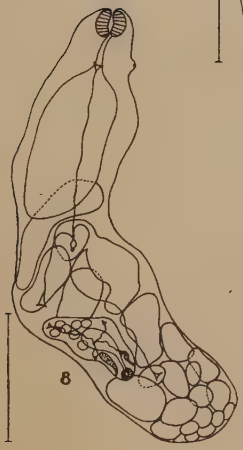
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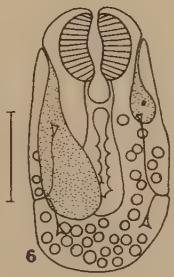
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INTRACELLULAR MICROÖRGANISM-LIKE BODIES IN THE TICK *DERMACENTOR VARIABILIS* SAY

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Pinkerton and Hass (1937), in a study of the microörganisms naturally occurring in *Dermacentor variabilis* collected in Massachusetts, noted, in the ovaries of all the ticks studied, numerous intracellular coccoid bodies staining dark blue with Giemsa and often appearing ring-shaped. Such bodies were also occasionally found in the lining cells of the alimentary tract, but not in any other tissue. Although many other kinds of microörganisms were found in some of the ticks, only these coccoid bodies occurred in all of the ticks.

The development of these bodies during the life cycle of *D. variabilis* forms the subject of the present communication.

MATERIAL AND METHODS

Adult ticks were collected near Princeton, New Jersey, and Woods Hole, Massachusetts, during the summer of 1937. From these, a number of laboratory-reared lines were started. All the stages were fed on guinea pigs and were kept during their fasting periods in tubes held over moist sand in an incubator at 25° C. Material for study was taken only from those lines which during two laboratory generations showed no microörganism-like bodies other than the coccoid bodies.

Entire ticks were fixed in Zenker or in Carnoy-Lebrun. After fixation the ticks were washed in 2 changes of water and were then stored for several weeks to several months in 70 per cent alcohol. They were prepared for sectioning by the method recommended by Slifer and King (1933) for grasshopper eggs. The ticks were placed in 70 per cent alcohol with iodine, then in 70 per cent alcohol, 80 per cent alcohol with 4 per cent phenol, 95 per cent alcohol, aniline, xylene, and xylene-paraffin. They were embedded in paraffin in the usual way. Enough of the paraffin block was then cut away to expose the tissue, and the block was soaked overnight in water. It was finally sectioned in the ordinary manner. By this method, good sections were obtained of larvae, engorged larvae, nymphs and recently molted adult females, as well as fair sections of engorged nymphs and of a partially engorged female. Entire males could not be sectioned satisfactorily.

Eggs of known age were first fixed in hot Carnoy-Lebrun and were then partially embedded in paraffin in the well of a culture slide. The

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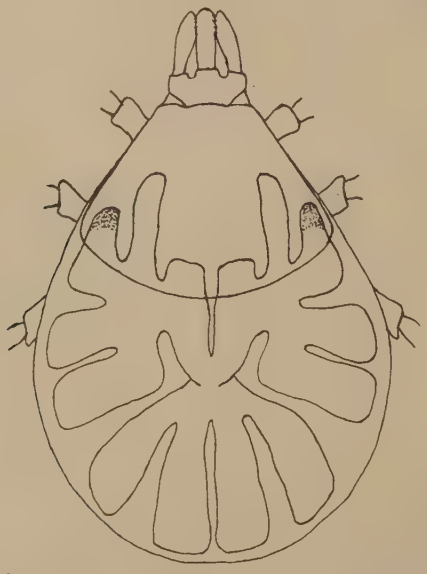
eggs were covered with Ringer's solution. Each egg was removed from its shell under a dissecting microscope, using needles ground to a fine point. The eggs were further treated in the same manner as entire ticks.

The sectioning of malpighian tubes and ovaries dissected out from adult ticks presented no difficulties.

All the sections were stained by Wolbach's (1919) Giemsa method. Complete serial sections of a large number of larvae and nymphs and of some eggs and adults were studied. Smears were also prepared and were stained both with Giemsa and with Gram's stain.

OBSERVATIONS

In *D. variabilis* larvae which were ready to feed, and hence at least 10 days old, masses of the very distinct dark purplish-blue coccoid bodies occurred only in the cells forming the anterior tip of the two more lateral anterior diverticula of the alimentary tract (Text-Fig. 1, Plate I, Fig. 1). The bodies varied in diameter from 0.4 to 0.8 μ. Many of



TEXT-FIG. 1. Diagrammatic dorsal view of larva of *D. variabilis*. The portions of the alimentary tract containing coccoid bodies are shaded.

them were clearly ring-shaped, the center of the ring appearing a pale pink. Some larvae apparently had larger numbers of the bodies than did others.

The coccoid bodies were present in engorged larvae in the same position as in the unfed larvae. In newly molted nymphs (Plate I, Fig. 2)

and also in engorged nymphs they were still restricted to the anterior portions of the two more lateral anterior diverticula of the alimentary tract. Figures suggesting division stages of the bodies were noted in an engorged nymph fixed three days after dropping from its host. At this stage of development the coccoid bodies were still confined to the anterior diverticula, but in freshly molted adult male and female ticks the bodies were numerous in the basal portions of the cells of all parts of the alimentary tract. In the adults they were slightly smaller than in larvae and nymphs (Plate I, Fig. 3). This fact, coupled with their great increase in number, suggests that rapid multiplication of the bodies occurred during the latter part of the quiescent pre-adult stage. One series of sections through a female, fixed on the 4th day after attachment to a guinea pig and already noticeably enlarged, showed numerous typical coccoid bodies scattered through the basal portions of the alimentary tract cells. The bodies were also found in sections of ovaries removed from egg-laying females about 3 weeks after engorgement. They were not found in any tissue other than the ovaries and the alimentary tract, thus confirming the observations of Pinkerton and Hass (1937).

The coccoid structures were difficult to find in the developing egg of *D. variabilis*. Enough stages were seen, however, to indicate the probable developmental cycle. When the embryos were 11–12 days old, typical coccoid bodies (Plate I, Fig. 4) were present in yolk cells located near the center of the yolk mass. At this stage, the ganglion of the tick embryo existed only as a ventral thickening of ectoderm, and the dorsoventral muscles had not yet appeared. When the embryos were 14–17 days old, the ganglion, dorsoventral muscles, and rectal sac were definitely formed. The coccoid bodies were still present only in the center of the yolk mass, just above the posterior tip of the ganglion. This was also their location when the tick embryos were 19–21 days old and when they were 23–25 days old and about ready to hatch. In very recently hatched larvae, in which the alimentary tract had not yet formed, no coccoid bodies could be found in the anterior dorso-lateral regions of the body. But in slightly older larvae, having a formed alimentary tract, coccoid bodies were present in the anterior tips of the lateral anterior diverticula, just as in larvae ready to feed.

The morphology of the coccoid bodies is adequately represented by the photomicrographs of Plate I. It is noteworthy that throughout the life cycle of the tick they did not vary greatly in size or appearance. With Giemsa, they stained a dark purplish blue, usually with a more or less distinct pale pink center. In the embryos, they were somewhat more lightly stained. In smears made from larvae and adult females they stained Gram positive.

Very similar bodies were found in sections of four larvae of *D. andersoni** (Plate I, Fig. 5). Here again, the ring-shaped dark purplish blue bodies were present only in the cells of the lateral anterior diverticula of the alimentary tract. They differed from the bodies in *D. variabilis* larvae only in being smaller (0.3–0.5 μ) and slightly more numerous, and in extending farther posteriorly. In sections of a few larvae of *Haemaphysalis leporis-palustris* no structures suggesting microörganisms were found.

DISCUSSION

The observations reported in the preceding section indicate that the coccoid bodies of *D. variabilis* pass through the following developmental cycle. They are present in the ovaries of adult females, whence they infect the eggs. During embryonic development they come to lie in the center of the yolk mass and they are still in this location at the time of hatching. When the alimentary tract becomes developed they appear in the cells of the lateral anterior diverticula. Here they remain, with possibly a slight increase in numbers, during the larval, engorged larval, nymphal, and early engorged nymphal stages. During the pre-adult stage they multiply and invade the epithelial cells of the entire alimentary tract. How they get into the ovary, and how they get from the center of the yolk mass to the anterior diverticula of the alimentary tract are two problems concerning which nothing is known.

The size and staining reactions of the coccoid bodies, their remarkable uniformity in appearance in all the stages of the tick, and their presence in a certain region of the yolk of the developing egg as well as in the alimentary tract and in the ovaries indicate that they are probably microörganisms rather than food storage or secretion granules. One would hardly expect the identical type of cytoplasmic granule to occur in a limited portion of the alimentary tract, in the ovaries, and in the center of the yolk mass of the embryo. They may be distinguished at a glance from the small dark brown granules resulting from the digestion of hemoglobin, as well as from the large, pink-stained food vacuoles which occur in the alimentary tract cells of fed ticks. Their intracellular habitat, their occurrence in all ticks of the species *D. variabilis* so far examined, and their hereditary transmission place them among the so-called intracellular symbionts (Buchner, 1930; Glaser, 1930).

The occurrence of non-pathogenic apparently hereditarily transmitted microörganisms in ticks was first described in some detail by Cowdry (1925). He found extremely pleomorphic Gram negative bodies in the malpighian tubes and ovaries of 16 species of ticks, among them *D. variabilis*. It has been impossible to confirm his results so far as this

* These larvae were descendants of adult ticks which Dr. R. R. Parker kindly sent me.

species is concerned. A special search of the malpighian tubes in a large number of serial sections of larvae and nymphs and in smears and sections from adult ticks has revealed nothing suggesting a microörganism, except in a few adults in which uniform rickettsiae-like bodies were noted. These results are in complete agreement with those of Pinkerton and Hass (1937), who found nonpathogenic rickettsiae in the malpighian tubes of only 3 out of 20 ticks examined. It is noteworthy that the photomicrographs given by Cowdry, especially his Fig. 6, which were taken from smears of whole larvae, show structures strongly suggesting the coccoid bodies, but no such bodies are to be found in his drawings made from sections through the malphighian tubes. The extreme pleomorphism figured by Cowdry suggests that he may have been dealing with different structures, some of them microörganisms and some not, rather than with different forms of a single microörganism.

Mudrow (1932) has given an extensive description of the intracellular symbionts of *Rhipicephalus sanguineus*, *Dermacentor reticulatus* and *Boophilus annulatus*, as well as some notes on the occurrence of symbionts in other species of ticks. The organisms were found in the malpighian tubes, ovaries, and eggs. They bear little resemblance to the coccoid bodies of *D. variabilis*. Probably different species of ticks have different symbionts. Pinkerton and Hass (1937), however, found typical coccoid bodies in the ovaries of *Rhipicephalus sanguineus*, whereas according to Mudrow, the organisms of *R. sanguineus* are straight or slightly curved rods.

The most recent work concerned with cytoplasmic microörganism-like inclusion bodies in ticks is that of Gregson (1938). He states that in the gut epithelium of the engorging *D. andersoni*, three types of inclusion bodies may be seen: purple coccoid granules (probably identical with the bodies described in the present paper), oval brown particles, and transparent globules. He then proceeds to deal only with the last mentioned type. These globules are not present in unfed larvae, nymphs, or adults. They appear in the alimentary tract soon after the tick begins to feed, and, as engorgement proceeds, they become progressively larger and more numerous. They are located chiefly at the distal ends of the cells and are eosinophilic. As the globules enlarge, the cells themselves also enlarge. These properties, as well as the photomicrographs published by Gregson, fit perfectly the food vacuoles of ticks. Roesler (1934) has made a thorough study of the process of digestion in several species of ticks. The ingested blood is rapidly hemolyzed in the lumen of the gut, but all subsequent stages of digestion occur intracellularly. The hemolyzed blood is taken up by the alimentary tract cells and appears as globular food vacuoles in the distal portions of the cells. The food vacuoles are acidophilic and increase in number and

size as engorgement proceeds. They are naturally present in small numbers or not at all in unfed ticks. As intracellular digestion proceeds, masses of dark brown excretory granules are formed. All these structures have been seen in the course of the study of the coccoid bodies. The inclusion bodies in phagocytic cells described by Gregson have also been seen. Their appearance suggests that they are food storage or secretion droplets.

SUMMARY

Small coccoid bodies, probably microörganisms, have been found in the epithelial cells of the anterior tip of the more lateral anterior diverticula of the alimentary tract in unfed and engorged larvae and nymphs of *Dermacentor variabilis*. These bodies are present throughout the lining cells of the alimentary tract of adult ticks, as well as in the ovaries and eggs. During embryonic development they lie in the center of the yolk mass, where they are still to be found at the time of hatching. The coccoid bodies were present in all ticks of this species examined. Bodies almost exactly like those of *D. variabilis*, and present in the same portion of the alimentary tract, were found in larvae of *D. andersoni*.

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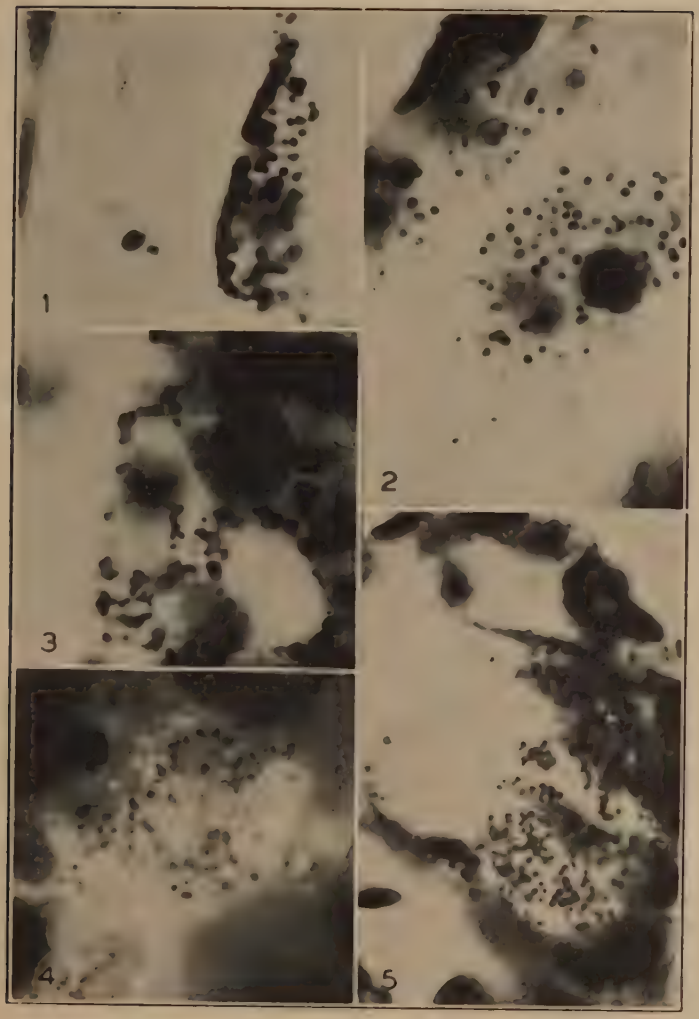

PLATE I

The coccoid bodies in:

FIG. 1. Larva of *D. variabilis;*
FIG. 2. Nymph of *D. variabilis;*
FIG. 3. Adult *D. variabilis;*
FIG. 4. 11 to 12 day old embryo of *D. variabilis;*
FIG. 5. Larva of *D. andersoni.*

All photomicrographs by J. Carlile from Giemsa stained sections. ×1420.

COMPARATIVE EFFICIENCY OF VARIOUS TECHNICS FOR THE DIAGNOSIS OF PROTOZOA AND HELMINTHS IN FECES

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INTRODUCTION

In a previous communication (1) a quantitative evaluation was presented of several technics for the diagnosis of protozoan cysts and helminths' eggs in feces. None of the concentration technics studied yielded as great a quantity of protozoan cysts per gram of original feces as did the direct iodine-stained fecal film. Nevertheless, due to a relatively greater removal of feces than of cysts, concentrations of varying degrees were obtained, depending on the technic used. Our zinc sulphate centrifugal floatation achieved a far greater concentration than any of the

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[1] We wish to express our indebtedness to Doctors Maud Loeber, Ruth Aleman and Herbert Weinberger, physicians in charge for the medical care of the children examined, and to the Mothers Superior and Father, superintendents and nurses in the institutions surveyed, for their helpful coöperation during this study. We likewise extend thanks to Mrs. Antonie Sawitz for assistance in preparing the charts summarizing our data.

other technics tested. However, it was not known what effect the utilization of the zinc sulphate technic would have in the clinical laboratory. The present report embodies this information. The findings are based on a comparative study of different technics for the recovery and diagnosis of both protozoan and helminthic infections in feces.

MATERIALS AND TECHNICS EMPLOYED

Materials Utilized

During the summer months of 1938, 271 freshly passed fecal specimens were obtained from 242 individuals in children's homes and in local clinics in New Orleans. In the first institution 117 fecal specimens were obtained from 45 boys and 48 girls, aged from 9 months to 7 years, and 16 fecal specimens from one male and 15 female workers in this home. In the second institution 51 fecal specimens were obtained from 50 boys, aged from 11 to 16 years. In the third institution, 64 fecal specimens were obtained from 63 boys, aged from 5 to 13 years. Twenty-three fecal specimens were obtained from 20 clinic patients, two males and 18 females, aged from 2 to 42 years.

Altogether 214 individuals were examined once, 27 twice and one, three times.

The great majority of the fecal specimens had been passed 1 to 2 hours before they were brought to the laboratory, and examination was begun less than 4 hours after the specimens were received. The great majority of the specimens were formed, only a few being semi-formed or diarrheic.

Description of Technics Tested

The following technics (referred to as numbered) were tested:

1. Direct fecal film, unstained and iodine-stained;
2. A similar fecal film prepared from cheesecloth-strained feces;
3. A similar fecal film prepared from wiregauze-strained feces;
4. Hematoxylin-stained direct fecal film;
5. Hematoxylin-stained suspension-serum fecal film;
6. Centrifugalization;
7. Zinc sulphate centrifugal floatation, followed by touch-removal;
8. Zinc sulphate centrifugal floatation, followed by loop-removal;
9. Hematoxylin-stained film prepared from material floated by zinc sulphate solution and removed by loop;
10. Brine centrifugal floatation with superimposed coverglass;
11. Zinc sulphate centrifugal floatation, with superimposed coverglass, carried out for the first 73 specimens studied.

Except for Technics 1 through 4, all of the preparations utilized a basic suspension of feces, made up as follows:

Five grams of freshly passed feces were weighed into a sieve fitted into a glass jar and having a brass screen base with 40 meshes to the

linear inch. Ten cc of physiological salt solution were mixed with the feces, and the mixture gently forced through the screen into the glass jar by means of a tongue depressor. Another amount of 10 cc of physiological salt solution was added, washing the debris remaining in the sieve of all fine particulate material. This screened mixture, homogenized by gentle manual rotation, provided a basic 1-in-5 fecal suspension.

Technic 1 (*direct fecal film, unstained and iodine-stained*). A representative fleck of feces, removed from the specimen, was thoroughly mixed on a broad slide (37×75 mm) with a drop of physiological salt solution. The resulting film was streaked out on the slide over an area approximately 20 by 40 mm. A clean coverglass was placed on one half of this film. To the uncovered portion a drop of D'Antoni's iodine stain (2) was added, thoroughly mixed with it, and, in turn, covered. The entire film was immediately examined for trophozoites, precysts and cysts of protozoa, as well as for eggs and larvae of helminths, with a compound binocular microscope having paired oculars 8 X and a 4 mm fluorite objective.

In carrying out this technic, as well as in all following, the time allowed for the examination of each slide and tabulation of the findings was limited to 10 minutes.

Technic 2 (*fecal film prepared from cheesecloth-strained feces*). An unstrained 1-in-5 suspension of feces in physiological salt solution was prepared and one portion was strained through one layer of cheesecloth, having 31 meshes to the linear inch. Twenty cmm of this material were pipetted onto a broad slide and mixed with an equal amount of physiological salt solution. The resulting film was prepared and examined as in Technic 1.

Technic 3 (*fecal film prepared from wiregauze-strained feces*). A second portion of the unstrained 1-in-5 fecal suspension utilized in Technic 2 was strained through brasswire gauze having 40 meshes to the linear inch. Twenty cmm were pipetted onto a broad slide and mixed with an equal amount of physiological salt solution. The resulting film was prepared and examined as in Technic 1.

Technic 4 (*hematoxylin-stained direct fecal film*). A representative fleck of the fecal sample was spread in a thin film on a clean 25×75 mm glass slide by means of a wooden toothpick. After fixation in hot Schaudinn's solution (60° C) for five minutes, Faust's iron-hematoxylin stain technic was employed (3).

The film was examined with a compound microscope having a 10 X ocular and 1/12 mm oil immersion objective.

Technic 5 (*hematoxylin-stained suspension-serum fecal film*). Ten cmm of fresh human serum were placed on a clean 25 x 75 mm slide. An equal amount of the basic 1-in-5 suspension was introduced onto the same slide from a conical-tipped pipette which was calibrated to 0.001 cc. The serum and suspension were mixed together with a polished needle and spread as a thin film over an area approximately 20 by 40 mm. Immediately the slide was inverted and immersed face-downwards into hot Schaudinn's fluid (60° C), in an attempt to bring all parts of the film into contact with the fixative at the same time. This was necessary to prevent drying and to eliminate scaling of the film. If scaling occurred, the procedure was repeated until a satisfactory film was obtained. The slide was held in the fluid until coagulation had occurred (approximately 5 seconds), then placed into a staining jar containing additional hot Schaudinn's fluid to complete fixation. The film was then stained as described in Technic 4. It was examined with a compound microscope having a 10 X ocular and a 1/7 oil immersion fluorite objective. In cases of doubtful identification the film was again examined under a 1/12 mm objective.

Technic 6 (*centrifugalization*). One cc of the basic 1-in-5 fecal suspension was pipetted into a calibrated Wassermann tube and tap water added to within one cm of the top. After centrifugalization for 45 seconds at a top speed of 2640

RPM the supernatant fluid was decanted, tap water again added, the sediment broken up by shaking, and the preparation recentrifugalized. This procedure was repeated twice, and the last supernatant fluid then poured off. D'Antoni's iodine stain was added to make up a total volume of 2 cc. The tube was well shaken and 0.1 cc of the stained suspension pipetted onto a slide. Examination was made with a compound microscope having a 12 X ocular and a 5 mm objective.

The centrifuge used for Technic 6, as well as in other centrifugalizations, was the International Clinical Centrifuge, in which the distance from the axis to the bottom of the tube is 13.5 cm. This centrifuge was selected as being generally employed in diagnostic laboratories and quite satisfactory for our purpose.

Technic 7 (zinc sulphate centrifugal floatation, followed by touch-removal). Two cc of the basic 1-in-5 fecal suspension were pipetted into a Wassermann tube. Tap water was added to within one cm of the top and the contents thoroughly mixed. The tube was centrifugalized for 45 seconds at 2640 RPM and the supernatant fluid poured off. Tap water was added to the sediment, which was meanwhile agitated in order to obtain a uniform mixture, and the tube centrifugalized as before. The procedure of washing was repeated until the supernatant fluid appeared clear. This usually required 2 or 3 centrifugalizations. After the last washing, approximately 1 cc of zinc sulphate solution of the specific gravity 1.180 was added to the packed fecal material. This solution is prepared by placing 331 grams of $ZnSO_4+7H_2O$ (granular U. S. P.) in a graduate cylinder and adding distilled water to the one liter mark. It is hereinafter referred to as "zinc sulphate solution." In order to mix the sediment thoroughly with the zinc sulphate solution, the tube was held with one hand near the top and was tapped near its bottom with a finger of the other hand. Zinc sulphate solution was then added to within ½ cm of the top of the tube and the mixture centrifugalized for 45 seconds. By means of a medicine dropper, zinc sulphate solution was again added, drop-by-drop, near the inside edge of the tube, until the meniscus was well rounded over the top of the tube. A clean broad slide was then superimposed onto the rounded meniscus, care being taken that the slide did not touch the top of the tube. The slide, with the fluid containing the removed surface-film material, was subsequently turned right-side-up and one drop of D'Antoni's iodine stain added. To insure uniform staining and to shift the material towards the center, the slide was rotated manually. A clean 22 mm square coverglass was placed on the stained preparation and the slide examined for protozoa and helminths with a compound microscope having a 10 X ocular and a 4 mm objective.

Technic 8 (zinc sulphate centrifugal floatation, followed by loop-removal). Two cc of the basic 1-in-5 fecal suspension were pipetted into a Wassermann tube and washed by centrifugalization, as described in Technic 7, until the supernatant fluid was clear. After final centrifugalization with zinc sulphate, the floated material was removed by means of a bacteriological loop 5 mm in diameter. In order that the surface film might not be unduly disturbed, the wire loop was carefully inserted vertically at one side of the tube beneath the meniscus, turned horizontally and then moved towards the center of the film. A loopful of floated material was then taken from this undisturbed portion of the film. The contents of the loop were transferred to a clean broad slide and the procedure repeated until three loopfuls had been obtained. One drop of D'Antoni's iodine stain was added to the removed material, and the slide rotated manually to insure a uniform staining and to shift the material towards the center. The preparation was covered with a 22 mm square coverglass and examined with a compound microscope having a 10 X ocular and a 4 mm objective.

Technic 9 (hematoxylin-stained film prepared from material floated by zinc sulphate solution and removed by loop). Two cc of the basic 1-in-5 suspension were centrifugalized and floatation was accomplished as described in Technic 7. Approximately 5 cmm of fresh human serum were introduced onto a 25×75 mm slide, and a loopful of material from the surface film of the Wassermann tube placed directly on it. The combined materials were mixed and spread with a polished needle over a 20×40 mm area, then treated as in Technic 5. Examination

was made with a compound binocular microscope having paired oculars 8 X and a 1/12 oil immersion fluorite objective.

Technic 10 (brine centrifugal floatation). Two cc of the basic 1-in-5 fecal suspension were pipetted into a Wassermann tube having a ground top. Tap water was added to within one cm of the top, and thoroughly mixed with the suspension by agitation. After the tube was centrifugalized for 45 seconds at a speed of 2640 RPM, the supernatant fluid was poured off. A small quantity of tap water was added, and the sediment broken up. More water was added to fill the tube to within one cm of the top, and the tube agitated again to mix the contents. The tube was recentrifugalized for 45 seconds. This procedure was repeated three or four times until the supernatant fluid was clear. After the last fluid was poured off, a small quantity of saturated sodium chloride solution (sp. gr. 1.200), afterwards referred to as brine, was added, and the tube tapped to break up the sediment. The tube was then completely filled with the brine, and a clean round 22 mm coverglass was superimposed. This coverglass was held in place by four metal fingers projecting upwards from a sleeve attached to the metal tube of the centrifuge, as an adaptation of Lane's direct centrifugal floatation technic (4). After 45 seconds of centrifugalization at 2640 RPM, the superimposed coverglass was transferred to a broad slide, and examined with a compound binocular microscope having paired 10 X oculars and a 4 mm objective.

Technic 11 (zinc sulphate centrifugal floatation with superimposed coverglass). Two cc of the basic 1-in-5 fecal suspension were pipetted into a Wassermann tube, the top of which had been ground flat. The supporting metal tube of the centrifuge was the same as that used in Technic 10. After washing the suspension by centrifugalization, as described in Technic 7, one cc of zinc sulphate was added, thoroughly mixed with the sediment, and the tube filled until the rim of the meniscus was level with the top of the tube. A clean round 22 mm coverglass was carefully placed on top of the brim-full tube, being held in place by the projecting metal fingers. After the preparation had been centrifugalized at 2640 RPM, for 45 seconds, the coverglass was carefully removed from the top of the tube with sharp vertical lift, and inverted. One drop of D'Antoni's iodine stain was added to the fluid on the coverglass, which was carefully rotated manually to insure uniform staining and shifting of the material towards the center. A clean broad slide was then superimposed on the coverglass and the entire preparation turned right-side-up. It was then examined with a compound microscope having a 10 X ocular and a 4 mm objective.

PRESENTATION OF DATA

The analysis of our data presented below is restricted to a comparative evaluation of the technics described above. For simplicity and to save space the findings are presented in tabular form. Only those cases have been included in which a definite diagnosis could be made.

TABLE 1.—*Comparative findings of protozoa by Technics 1, 2 and 3*

Species	Number of infections diagnosed by technics			
	1	2	3	1, 2, 3
E. histolytica	45	43	43	64
E. coli	99	106	107	126
E. nana	105	97	94	124
Iodamoeba bütschlii	5	1	2	5
Giardia lamblia	58	53	50	63
Chilomastix mesnili	29	33	33	39
Trichomonas hominis	4	4	3	6
Total	345	337	332	427

TABLE 2.—*Comparative results for the diagnosis of protozoa and helminths from 189 fecal specimens by Technics 1–10*

Species	Total number of infections found by technics 1–10	Number (and *percentage in italics*) found by respective technics																	
		1		2		3		4		5		6		7		8		9		10	
E. histolytica ..	94	31	*33*	33	*35*	36	*38*	37	*39*	17	*18*	36	*38*	65	*69*	65	*69*	34	*36*		
E. coli	130	72	*55*	80	*62*	81	*62*	82	*63*	57	*43*	89	*68*	120	*92*	118	*91*	85	*65*		
E. nana	155	68	*44*	65	*42*	67	*43*	115	*74*	78	*50*	62	*40*	108	*70*	110	*71*	67	*43*		
Iodamoeba	16	3	*19*	1	*7*	1	*7*	5	*31*	1	*7*			7	*44*	6	*38*	3	*19*		
Dientamoeba ...	3									3	*100*										
Giardia	39	24	*62*	19	*49*	20	*51*	23	*59*	17	*44*	17	*44*	29	*74*	34	*87*	19	*49*		
Chilomastix ...	47	20	*43*	23	*49*	23	*49*	32	*68*	12	*26*	20	*43*	32	*68*	33	*70*	19	*40*		
Trichomonas ...	2	1	*50*							1	*50*										
All protozoa ...	486	219	*45.1*	221	*46.5*	228	*46.9*	294	*60.5*	186	*38.3*	224	*46.1*	361	*74.3*	366	*75.3*	227	*46.7*		
Ascaris	2	2	*100*	2	*100*	2	*100*	1	*50*			1	*50*	1	*50*	2	*100*	1	*50*	2	*100*
Enterobius	16							1	*6*					13	*81*	13	*81*	2	*13*	11	*69*
Whipworm	32	2	*6*	1	*3*	2	*6*	1	*3*	1	*3*	3	*9*	25	*78*	28	*88*	4	*13*	31	*97*
Hookworm	5											1	*20*	4	*80*	5	*100*			4	*80*
Strongyloides ..	3											1	*33*			2	*67*	1	*33*		
All helminths ..	58	4	*6.9*	3	*5.2*	4	*6.9*	3	*5.2*	1	*1.7*	6	*10.3*	43	*74.1*	50	*86.2*	8	*13.8*	48	*82.8*
Total protozoa and helminths	544	223	*41.0*	224	*41.2*	232	*42.6*	297	*54.6*	187	*34.4*	230	*42.3*	404	*74.3*	416	*76.5*	235	*43.2*		

Protozoan Findings With Different Direct Iodine-stained Films

All of the 271 fecal specimens in this series were examined by Technics 1, 2 and 3. The protozoan findings obtained from these technics (including trophozoites, precysts and cysts) are summarized in Table 1.

Comparative Results Obtained by Use of Different Technics

In Table 2 are summarized the results obtained from the examination of 189 fecal specimens by Technics 1 through 10.

Number of Infections With Only Unencysted Protozoa

Detection of infections with only trophozoites and/or precysts was not anticipated from the examination of films obtained by any of the concentration technics, although viable *Giardia* trophozoites were occasionally found by Technics 7 and 8. The number of infections in which only trophozoites and precysts were seen is summarized in Table 3.

TABLE 3.—*The number of infections in which only trophozoites and/or precysts were found*

Species	Number found by technics:				
	1	2	3	4	5
E. histolytica	2	5	5	3	2
E. coli	2	2	1	3	1
E. nana	4	7	6	6	3
Chilomastix	2	3	3	1	0
Trichomonas	1	0	0	0	1

Combination of Technics to Increase the Diagnostic Yield

In routine clinical laboratory examinations a combination of technics is frequently employed in order to increase diagnostic efficiency. Table 4 shows the number of infections diagnosed when a combination of Technics 1 and 4, 1 and 6, 4 and 6, 1 and 8, 4 and 8, 1, 4 and 8 (for protozoa only) and 1, 6 and 10 (for helminths only) was employed.

The information in Tables 2, 3 and 4 is graphically presented in charts, Figs. 1, 2 and 3.

DISCUSSION

Introductory Remarks

In the preliminary report on our experimental quantitative evaluation of technics for the recovery and diagnosis of protozoa and helminths in feces (1), we anticipated that, after adequate tests had been made, we might be able to recommend a technic or combination of technics for both protozoa and helminths which is simple and at the same time more dependable than those used in the diagnostic laboratory. Our present study warrants a fulfillment of this promise.

Technics 1, 2 and 3 were employed in order to test the effect on diagnosis of straining a fecal suspension through cheesecloth or wire gauze.

TABLE 4. *Diagnostic results obtained by combinations of technics*

Species	Total number found by all technics	Number (and *percentage in italics*) by combined technics: 1 and 4		1 and 6		4 and 6		1 and 8		4 and 8		1, 4 and 8		1, 6 and 10	
E. histolytica	94	48	*51*	45	*48*	54	*57*	70	*75*	71	*76*	76	*81*		
E. coli	130	91	*70*	98	*75*	99	*76*	119	*90*	122	*94*	130	*100*		
E. nana	155	128	*83*	87	*56*	124	*80*	117	*76*	137	*88*	149	*96*		
Iodamoeba	16	8	*50*	3	*19*	5	*31*	9	*56*	10	*63*	13	*81*		
Dientamoeba	3														
Giardia	39	28	*72*	26	*67*	26	*67*	36	*92*	35	*90*	38	*97*		
Chilomastix	47	35	*75*	29	*62*	36	*77*	35	*75*	37	*79*	39	*83*		
Trichomonas	2	1	*50*	1	*50*			1	*50*			2	*100*		
All protozoa	486	339	*69.8*	289	*59.5*	344	*70.8*	387	*79.6*	412	*84.8*	447	*92.0*		
Ascaris	2	2	*100*	2	*100*	1	*50*	2	*100*	2	*100*			2	*100*
Enterobius	16	1	*6*			1	*6*	13	*81*	13	*81*			11	*69*
Whipworm	32	2	*6*	1	*13*	3	*9*	28	*88*	28	*88*			31	*97*
Hookworm	5			1	*20*	1	*20*	5	*100*	5	*100*			4	*80*
Strongyloides	3			1	*33*	1	*33*	2	*67*	2	*67*			1	*33*
All helminths	58	5	*8.6*	8	*13.8*	7	*12.0*	50	*86.2*	50	*86.2*		-	49	*84.5*
Total protozoa and helminths	544	344	*63.2*	297	*54.6*	357	*65.5*	437	*80.3*	462	*84.9*				

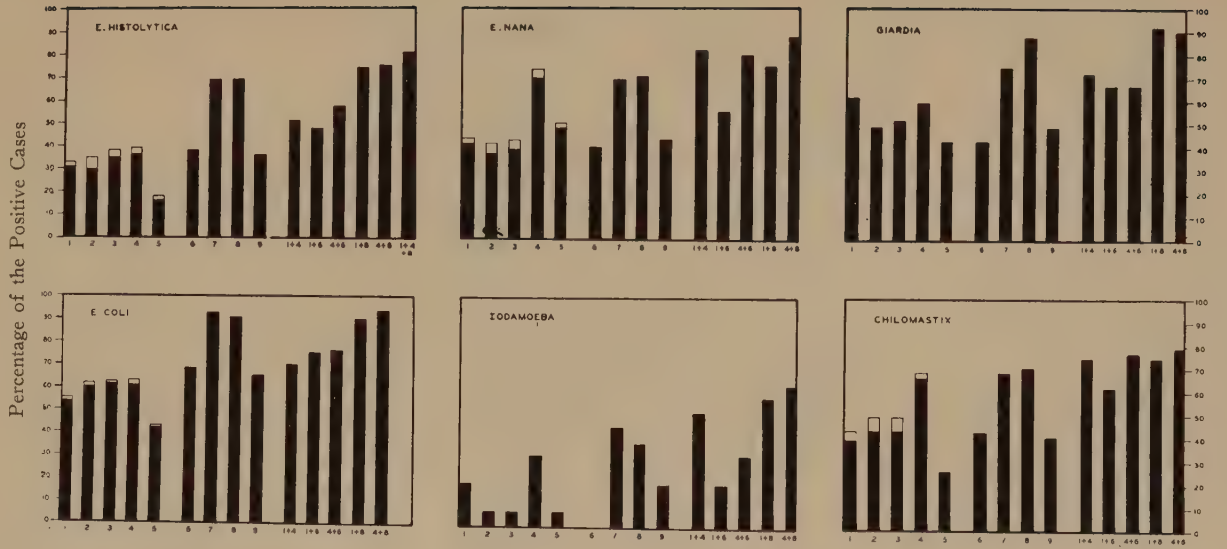

FIG. 1. Efficiency of various technics for the detection of protozoan species in feces, based on the examination of 189 fecal specimens. The ordinates in each block diagram represent the percentages of the total positives diagnosed by Technics 1 through 9. The abscissa figures refer to the technics employed, viz.: 1, direct iodine-stained fecal films; 2, cheesecloth-strained fecal suspension; 3, wire-gauze-strained fecal suspension; 4, direct hematoxylin-stained fecal film; 5, hematoxylin-stained suspension-serum; 6, centrifugalization; 7, zinc sulphate, followed by touch removal; 8, zinc sulphate, followed by loop removal; 9, zinc sulphate, followed by loop removal and hematoxylin staining.

The black columns indicate the percentages of diagnosed specimens in which cysts were found; the white blocks topping the black columns represent the percentages in which only trophozoites and/or precysts were found.

Technic 1 (direct, unstrained fecal film) served as a control for the other two. Table 1, which summarizes our protozoan findings with these three technics, shows no significant effect produced by either type of straining, although for the majority of species no single technic yielded more than 75 to 80 per cent of the total positive diagnoses obtained by all three of these technics. It is probable that some protozoa and helminths may have been removed by straining, but the greater visibility obtained by the removal of large particulate material in the feces rendered discovery and

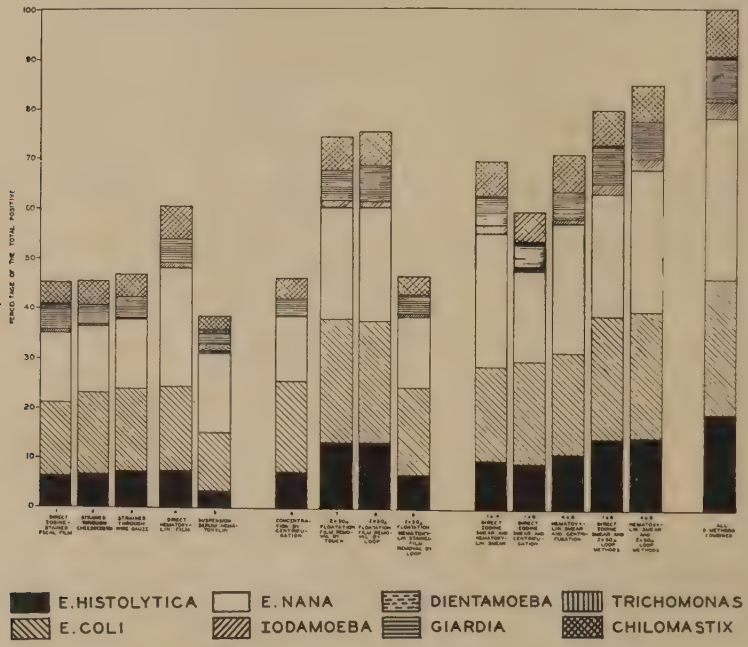

FIG. 2. Comparison of the summarized findings of protozoa in 189 fecal specimens by Technics 1 through 9. The ordinate and abscissa are the same as for Fig. 1. The last bar represents all methods combined.

diagnosis of the parasites easier and thus compensated for the loss. This test demonstrated that a strained 1-in-5 fecal suspension was diagnostically not inferior to the direct fecal film, and thus made it safe for us to use a basic 1-in-5 fecal suspension for hematoxylin-serum and concentration technics. Nevertheless, we do not wish to imply that in routine diagnosis a strained fecal suspension should be used as a substitute for the simpler, direct fecal film.

In addition to the first three technics, already considered, Technic 4 (direct hematoxylin-stained fecal film) and Technic 5 (hematoxylin-

stained suspension-serum film) are diagnostic methods which do not aim at concentration. Since Technic 5 was used in our preliminary quantitative study, it seemed necessary to compare its diagnostic efficiency with the standard hematoxylin-stained direct fecal film.

Technics 6 through 11 aim at concentration. Centrifugalization (Technic 6) is a routine method employed in practically all clinical laboratories. Wassermann tubes were used in place of larger-sized centrifuge tubes, because they were easier to handle, were cheaper, and at the same

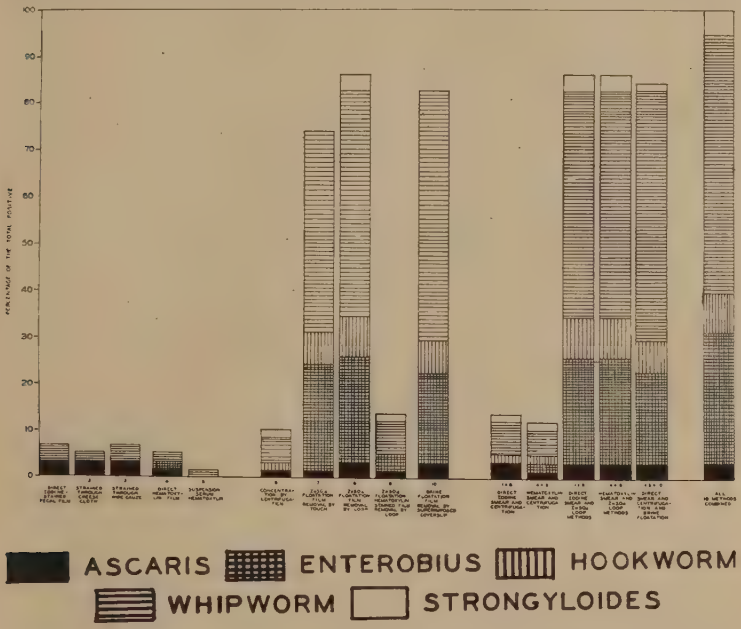

FIG. 3. Comparison of the summarized findings of helminths in 189 fecal specimens by Technics 1 through 10. The ordinate and abscissa are the same as for Fig. 1 for Technics 1 through 9. Technic 10 is an adaptation of Lane's D.C.F. The last bar represents all methods combined.

time provided an adequate amount of fecal material for diagnostic use. One cc of the 1-in-5 fecal suspension contains approximately 0.2 gm of feces, which previous quantitative tests have shown to be the optimum amount for 2 or 3 centrifugalizations. A smaller amount is inadequate and a larger amount would require more centrifugalizations to render the supernatant fluid clear. Certain eggs of helminths are thrown down out of a fecal suspension at considerably lower speeds and in a much shorter time than were employed by us. However, we found that protozoan cysts, especially those of *Giardia,* required slightly less than 45 seconds

at top speed (2640 RPM) of our centrifuges. Since we were attempting to recover all types of protozoan cysts as well as eggs of helminths, and since the longer period of centrifugalization did not result in loss of the heavier diagnostic material, we used speed and time factors slightly in excess of the requirement for the lightest organisms to be recovered. In utilizing Technic 6, as well as all other technics requiring centrifugalization, we have followed this rationale.

Brine floatation, originally devised by Bass (5) in 1906, is a simple, efficient procedure, used in many clinical laboratories and in the field, for the recovery of helminths' eggs. Lane (4), in 1922, devised a refinement of the combined centrifugalization and brine floatation technics which Bass (6) developed in 1909. Lane's D. C. F. technic is recognized to be both qualitatively and quantitatively very efficient for the concentration of helminths' eggs. Our brine centrifugal floatation (Technic 10) was an adaptation of Lane's.

Because brine sufficiently concentrated to float protozoan cysts shrinks them so badly that they are undiagnosable, an attempt was made by us, soon after we began our investigation on diagnostic technics, to find a medium, as efficient as brine, which would float both protozoan cysts and helminths' eggs, without impairing their diagnostic properties. Tests were made with sugar solutions, glycerin, ethylene glycol and hydrochloric acid-ether, all of which produced high concentration of the parasites, but invariably shrank the protozoan cysts, so that they could not be specifically diagnosed. In addition, the following metal salts in aqueous solution were tested: sodium sulphate, ferric ammonium sulphate and aluminum potassium sulphate. All were found to be unsatisfactory. Copper sulphate solution with a specific gravity of 1.180, modified from the specific gravity of 1.200 recommended by Khaustov (7) in 1935, was found to be quite satisfactory for floating eggs and cysts which were only slightly shrunken; but a black gummy precipitate was formed when iodine solution was added to stain the cysts, making diagnosis difficult or impossible.

In searching for a solution which had a high specific gravity, which was colorless, and which did not combine with iodine to form a precipitate, zinc sulphate in aqueous solution was investigated. In addition to having these properties, it caused no appreciable shrinkage of either cysts or eggs and no loss in their viability within a period of an hour or more. Solutions of this salt varying in specific gravity from 1.150 to 1.250 were tested, and a specific gravity of 1.180 was found to be eminently satisfactory for floating both cysts and eggs in a minimum amount of fecal debris. This specific gravity of zinc sulphate solution has, therefore, been utilized in our present study for comparison with other concentration technics.

We had now demonstrated that zinc sulphate solution having the specific gravity of 1.180 was an appropriate medium for the floatation of cysts and eggs for diagnosis. Likewise, we had previously found (1) that superimposed coverglass-removal, which we adapted from Lane's D. C. F. (4), actually produced a concentration up to 400-fold. However, we did not know how this method compared in practical efficiency with other methods for removal of the surface film. Bass (6) used a pipette to remove the surface film from his centrifugal floatation preparations. Kofoid and Barber (8), in 1918, introduced the loop-removal of eggs from brine. Subsequently Willis (9), in 1921, recommended a touch technic, in which a superimposed slide made contact with the surface of a brine suspension. Neither Kofoid and Barber nor Willis combined centrifugalization with floatation.

Our tests included a comparison of touch-removal (Technic 7), loop-removal (Technic 8) and superimposed coverglass-removal of the surface film following centrifugal floatation in zinc sulphate (Technic 11). The first 73 specimens were examined by all three of these technics, but the findings for protozoa indicated that the touch and loop methods had the same practical efficiency as the superimposed coverglass removal. Hence, in all subsequent examinations, Technic 11 was discontinued, since it required specially prepared Wassermann tubes and metal supports in order to carry out the modified Lane technic and since it was more laborious than the other two methods. However, in case of a quantitative study of either protozoan cysts or helminths' eggs, our data (1, 10) indicate the greater accuracy of the superimposed coverglass method.

The hematoxylin-stained films, obtained from material floated from zinc sulphate and removed by loop (Technic 9), were prepared in order to have permanent preparations from the zinc sulphate concentrates. These films were examined in order to discover their relative diagnostic value. Our results confirm the conclusion reached by Nickel (11), that "thus far this technic has not been sufficiently perfected to recommend it for routine examination in the clinical laboratory, since it requires considerable technical detail and great care."

Table 2 provides a comprehensive summary of the yields of protozoa and helminths obtained by Technics 1 through 10. The findings for six of the protozoa are also graphically presented in Fig. 1, while the data for all eight species of protozoa are summarized in Fig. 2. The relative efficiencies of each technic will be considered separately for each protozoon recovered.

Protozoan Findings

Endamoeba histolytica. No significant difference was found in the yields by Technics 1, 2, 3 and 4, although some positive diagnoses made

by each of these was missed by one or more of the others. The hematoxylin-stained direct fecal films provided a one per cent higher incidence than the highest yield obtained by the iodine-stained films. The hematoxylin-stained suspension-serum film was diagnostically inferior to the first four technics, not only for *E. histolytica* but also for the other protozoa recovered, with the exception of *Dientamoeba*.

The number of cases with *E. histolytica* detected by centrifugalization (Technic 6) was within the same range as in Technics 1, 2, 3 and 4, although a slightly higher number was diagnosed from fecal material containing cysts.

With zinc sulphate the number of positive cases of *E. histolytica* was almost twice that of the direct fecal films or centrifugalization. This indicates the superiority of zinc sulphate and its particular value in diagnosing fecal specimens containing relatively few cysts. Insofar as the detection of *E. histolytica* is concerned, there appears to be no choice between touch-removal and loop-removal.

Endamoeba coli. It is interesting and apparently significant, that of the total number of *E. coli* positives diagnosed a much higher percentage was found by each of the first nine technics than was the case for *E. histolytica*. This was possibly due to a considerably greater number of *E. coli* cysts present in the specimens examined. Even with the higher percentage of *E. coli* cases detected by the first six technics, each of the zinc sulphate methods diagnosed approximately 50 per cent more, and more than 90 per cent of the total number.

Endolimax nana. For this species the hematoxylin-stained direct fecal films yielded approximately 60 per cent more positive specimens, and the serum-hematoxylin films provided a slightly higher yield than did the direct iodine-stained films. It is suggested that this superiority of the hematoxylin films may be due to the minute size of *E. nana*, so that the organism was more readily found in the hematoxylin films, which were examined under oil immersion. The same tentative explanation is offered for the slightly higher yield of the direct hematoxylin film as compared with the zinc sulphate concentration technics.

Iodamoeba bütschlii. The total net number of positives detected by all technics was too small to warrant any definite evaluation of the several technics, although the highest percentage of positives was found by the zinc sulphate technics.

Dientamoeba fragilis. This organism was not diagnosed from the direct iodine-stained films, or from the concentrates, and was found only three times on hematoxylin-serum films. However, by employing considerably more than ten minutes on each film, it was diagnosed on two hematoxylin-stained direct fecal films and on six additional hematoxylin-serum films. In this small series of recoveries of this organism any explanation of the discrepancies would be hazardous.

Giardia lamblia. In no specimen were trophozoites alone recovered. The direct iodine-stained film was slightly, although probably not significantly, superior to the other direct fecal films. Centrifugalization was definitely inferior and the zinc sulphate concentrates superior to the direct films.

Chilomastix mesnili. For this organism both the hematoxylin stained direct fecal film and the zinc sulphate technics detected a markedly higher number of positives than did any of the other technics.

Trichomonas hominis. The two positive specimens were discovered only on direct films (Technics 1 and 5).

Comparative Findings on the Protozoa Recovered in This Study

Of the 486 protozoan positives diagnosed from the 189 specimens examined by Technics 1 through 10, slightly less than one-half of the number was found by each of the iodine-stained films, three-fifths by the direct hematoxylin technic, about two-fifths by the serum-hematoxylin technic, slightly less than one-half by centrifugalization and three-fourths by each of the zinc sulphate technics. As had been anticipated, the cysts recovered by brine floatation were too badly shrunken for identification. The high total findings obtained from the hematoxylin-stained direct fecal films are due almost entirely to the high yields of *E. nana* and *Chilomastix* by this technic.

When this study was undertaken it was realized that a maximum of ten minutes for examination of films prepared by any of these technics was probably too short a period for recovery and diagnosis of all species present in the film. However, this limit of time was arbitrarily set, because it is the usual amount of time devoted to this examination in the clinical laboratory, and, if adhered to by all members of our research group, would provide some comparative index of the relative efficiencies of the several technics under practical laboratory conditions. Furthermore, this handicap of time has convincingly demonstrated the ease and accuracy with which diagnosis of protozoan cysts can be made by the zinc sulphate centrifugal floatation technics.

Helminthic Findings

Ascaris lumbricoides. Only two positives were diagnosed.

Enterobius vermicularis. Of the 16 positives diagnosed from fecal material, none were discovered on the iodine-stained films, one on the hematoxylin-stained direct film, none by centrifugalization, 13 by each of the two zinc sulphate technics and 11 by brine floatation.

While the zinc sulphate technics yielded a higher percentage of positives than brine floatation, neither of these methods provides an accurate index of *Enterobius* infection in a population group. This statement has been conclusively substantiated by members of our own research group

(12), who have shown that the NIH cellophane-tipped anal swab (13) detects more than five times as many cases as can be diagnosed by zinc sulphate centrifugal floatation of fecal material.

Whipworm. The direct fecal films discovered only a small percentage of the total positives diagnosed. Both of the zinc sulphate technics yielded a significantly high number of positives, although loop-removal was found to be more efficient than touch-removal. However, for whipworm eggs, we have found brine centrifugal floatation to be slightly superior to the touch-removal and loop-removal with zinc sulphate. It is possible that zinc sulphate floatation with superimposed coverglass might be as efficient as, or more efficient than, the brine centrifugal floatation. As yet we have no adequate information on this point.

Hookworm. All of the infections diagnosed were very light, as demonstrated by the fact that they were detected only by concentration. All five positives were diagnosed by loop-removal, four each by touch-removal and brine centrifugal floatation, and one by centrifugalization.

Strongyloides. Three very light infections were diagnosed: one by centrifugalization, two by loop-removal, one on a hematoxylin-stained film obtained by loop-removal, and none by brine. Although a negative diagnosis with zinc sulphate concentration does not exclude the possibility of infection with this parasite, the ability to concentrate *Strongyloides* larvae by zinc sulphate adds a valuable diagnostic aid in this infection.

Comparative Findings on the Helminths Recovered in this Study

It is evident from the summarized total for helminths at the bottom of Table 2, graphically represented in Fig. 3, that the direct fecal films failed to discover any considerable part of the helminthic infections. This is especially understandable in view of the fact that practically all were light infections. Although it is possibly surprising that centrifugalization had an efficiency of only about one and one-half times that of the direct films, a preliminary quantitative study on hookworm eggs (Sawitz) has indicated that an actual concentration of only 1.5 to 2.0 may be obtained after three centrifugalizations. Whereas the direct films detected one-sixteenth of the total infections and centrifugalization one-tenth, brine detected four-fifths, zinc sulphate with touch-removal three-fourths, and zinc sulphate with loop-removal nearly nine-tenths of the total yield. Loop-removal is at least as efficient as brine for the recovery of helminths' eggs and has the advantage of concentrating *Strongyloides* larvae, usually in a diagnosable condition.

Data on the Recovery of Other Species of Helminths

Not included in the tabulated summary (Table 2) or in Fig. 3 are the findings of *Heterodera radicicola* eggs. Although *Heterodera* is not a

parasite of man or other animals, this nematode infects vegetables consumed by man and from time to time its eggs are recovered from human feces, frequently confusing diagnosis because of its partial resemblance to hookworm and pinworm eggs. Of the 271 fecal specimens examined, 51, or 19%, contained these eggs. None were detected by Technics 1 through 5, two by centrifugalization, 18 by touch-removal, 34 by loop-removal, and 47 by brine floatation.

Although we discovered no infection of *Schistosoma mansoni*, *Hymenolepis nana* or *Diphyllobothrium latum* in this series of specimens, in routine laboratory examinations we have had an opportunity to observe the high efficiency of zinc sulphate centrifugal floatation for the recovery of eggs of these three helminths. In these observations, as well as those made on *Heterodera* eggs, a high concentration of unshrunken and otherwise easily diagnosable eggs was obtained.

Recovery of Arthropods in Feces

In 9 specimens of the 271 examined in our series, mites, usually *Tyroglyphus* sp., were discovered in the films. None were obtained by Technics 1 through 6, six each by touch-removal and loop-removal and one by brine floatation.

Technics for the Detection of Unencysted Protozoa

As yet we do not know of any concentration or enrichment technic other than culture methods available for the identification of amebic trophozoites or precysts. As a result of the necessary manipulations in the concentration technics, mechanical, physical and chemical interference with the activity of the unencysted, and hence unprotected, organisms, commonly destroys or distorts amebae, or usually stimulates encystation of *Giardia* and *Chilomastix*. Thus, in any practical diagnostic procedure, it is necessary to use one of the technics which do not effect concentration and which preserve these stages of the protozoön in a diagnosable condition. Table 3 provides an analysis of the findings in which diagnosis was based solely on trophozoites or precysts, because in these specimens encysted organisms were not recovered by any technic. Hence failure to utilize direct fecal films would have resulted in missing these infections. Insofar as *E. histolytica, E. coli, E. nana, Chilomastix* and *Trichomonas* are concerned, there appears to be no especial superiority of any of these technics, but for *Dientamoeba* our present study shows the advantage of using a hematoxylin film for diagnosis. It is interesting (see Table 3) that the manipulations necessary for preparing a homogeneous suspension of the fecal specimen in physiological salt solution and straining it through cheesecloth or wire gauze do not impair the diagnostic properties of the encysted stages of these protozoa.

Efficiency Resulting from a Combination of Technics

While brine floatation is simple and is efficient for most species of helminth eggs, it renders protozoa undiagnosable. In a critical discussion of his D. C. F. technic Lane (14), in 1925, suggested that protozoologists should consider the advantages of direct centrifugal floatation for the diagnosis of protozoa present in feces. We believe that our zinc sulphate technic is the first one to achieve Lane's suggested goal, since zinc sulphate provides a high concentration of protozoan cysts in a condition very satisfactory for accurate diagnosis, and the concentration of helminth eggs is at least as efficient as Lane's D. C. F.

Since no one technic, by a single examination, as tested by us and by previous investigators, provides an optimum yield of all protozoa and helminths present in a fecal specimen, it is necessary either to perform repeated examinations using a single technic, or to combine two or more technics which promise the best results. While repeated examination of the same fecal specimen or of several specimens from the same patient by a single technic may achieve the desired end, a simpler way is to select a combination of the smallest number of technics which will approach 100 per cent efficiency.

In any combination it is essential to select one of the direct fecal films, in order to detect protozoa in specimens containing only unencysted organisms. If one of these technics is combined with zinc sulphate, a yield is obtained which is appreciably higher than either of the two methods alone. As demonstrated in Table 4, this holds true for protozoa as well as for helminths.

The total yields for protozoa by combining Technics 1 and 4, 1 and 6, 4 and 6, 1 and 8, and 4 and 8 are graphically presented in Fig. 2, for comparison with the yields of each technic alone and with the total yields by a combination of all methods studied. Thus, if any two technics are combined, the highest diagnostic efficiency for protozoa is achieved by a combination of the hematoxylin-stained direct fecal film and zinc sulphate concentration by loop-removal (85% of the total yield), and the second best combination consists in the iodine-stained direct fecal film and the loop-removal technic (80%).

The total yields for helminths by combining Technics 1 and 4, 1 and 6, 4 and 6, 1 and 8, 4 and 8, and 1, 6 and 10 are graphically presented in Fig. 3, for comparison with the yields of each technic alone and with the total yields by all technics. A combination either of Technics 1 and 8 or of 4 and 8 yields an efficiency of 86 per cent of the total yield obtained by all technics studied.

When both protozoa and helminths are considered (Table 4, bottom), the two highest percentages of the total infections found by all technics result from a combination of Technics 1 and 8 (80%) and of 4 and 8 (85%).

Technic 8 (zinc sulphate centrifugal floatation, followed by loop-removal) was selected instead of Technic 7 (zinc sulphate, followed by touch-removal) for combination with other technics, because of its higher efficiency in detecting *Giardia* cysts, whipworm eggs and *Strongyloides* larvae.

Thus a choice lies between a combination of Technics 1 and 8, and 4 and 8. Diagnosticians who have previously used the iodine-stained direct fecal films will probably prefer the former combination, while those who for one reason or another have a preference for the hematoxylin-stained direct film may continue to use this in combination with Technic 8. We wish to point out that neither the iodine-stained direct film nor the hematoxylin-stained direct film will by itself or in combination with centrifugalization or with brine centrifugal floatation, provide as high a percentage of positive diagnoses as either Technic 1 or 4 combined with 8. Moreover, if only a single technic is used, negative diagnosis is not as reliable as if Technic 1 or 4 had been combined with 8.

Application of the Zinc Sulphate Technic to Different Population Groups

The principles underlying zinc sulphate centrifugal floatation indicate that it is applicable to parasitological diagnosis of feces of any individual in any area. In our series of cases, having a high incidence of protozoan infections in individuals passing large numbers of the organisms, we practically doubled our percentage of diagnoses. In other population groups, with a lower incidence and especially in individuals passing relatively few protozoa, the diagnostic yield obtained by the use of this technic will undoubtedly be comparatively greater. Thus, in our recent examination of 166 fecal specimens from 86 medical students, three were found positive for *Endamoeba histolytica* by direct film examination, while the number of stools positive for *E. histolytica* by zinc sulphate floatation was fourteen, or nearly a five-fold increase. In our series of cases having a low infection incidence with helminths, in individuals passing small numbers of eggs (or larvae), the diagnostic yield was increased about twelve-fold by zinc sulphate compared with the direct fecal film. In population groups more heavily infected with helminths a comparatively smaller diagnostic increase would be anticipated.

Effect of Findings on the Interpretation of Parasitological Survey Data

In organizing and carrying out our investigations on parasitological examination of feces, our emphasis and efforts have been primarily directed towards the development of more efficient technics for use in the clinical laboratory. However, our findings have an equally significant bearing on parasitological surveys, including both those conducted in the past and those to be undertaken in the future. Now that a relatively simple and highly efficient concentration technic is available for com-

bination with the iodine-stained or hematoxylin-stained direct fecal film, survey workers have a means of carrying out field investigations with an assurance of much greater efficiency and reliability in their results than has heretofore been possible.

CONCLUSIONS

On the basis of our preliminary and present studies, as well as practical experience in our diagnostic laboratory, we are in a position to recommend zinc sulphate centrifugal floatation, followed by loop-removal for diagnosis of protozoan cysts and helminths' eggs, as well as for larvae of *Strongyloides* and hookworm present in fecal specimens. This technic must be supplemented by either iodine-stained or hematoxylin-stained direct fecal films to provide a safeguard in cases where only trophozoites or precysts of protozoa are present in the fecal specimen, and by the use of the NIH (cellophane-tipped) anal swab for accurate diagnosis of *Enterobius* infections.

SUMMARY

1. In order to determine their practical efficiency, various technics used for the discovery and diagnosis of protozoa and helminths present in feces have been critically tested on 271 fresh fecal specimens obtained from 242 individuals in children's homes and in medical clinics in New Orleans.

2. The following technics have been tested:

(1) direct fecal film, unstained and iodine-stained;
(2) a similar fecal film prepared from cheesecloth-strained feces;
(3) a similar fecal film prepared from wiregauze-strained feces;
(4) hematoxylin-stained direct fecal film;
(5) hematoxylin-stained suspension-serum fecal film;
(6) centrifugalization;
(7) zinc sulphate centrifugal floatation, followed by touch-removal;
(8) zinc sulphate centrifugal floatation, followed by loop-removal;
(9) hematoxylin-stained film prepared from material floated by zinc sulphate and removed by loop;
(10) brine centrifugal floatation with superimposed coverglass;
(11) for the first 73 specimens studied, zinc sulphate centrifugal floatation, with superimposed coverglass.

3. An analysis of the data obtained from this study has demonstrated that:

(1) Straining of a fecal suspension through cheesecloth or wiregauze does not impair the diagnostic qualities or reduce the

diagnostic yield of parasites present in the fecal specimen. This knowledge has permitted the use of a strained basic fecal suspension to compare concentration technics.

(2) Trophozoites and precysts of intestinal protozoa were seldom found and then almost exclusively in direct fecal films.

(3) Iodine-stained or hematoxylin-stained fecal films detected only about 40 per cent of the *Endamoeba histolytica* infections found, and only 46 to 60 per cent of all species of protozoa. These same technics recovered less than ten per cent of the helminths.

(4) Centrifugalization alone has not been found to produce significantly higher percentages of diagnosis than the direct fecal films.

(5) Zinc sulphate centrifugal floatation, followed by touch-removal or loop-removal, doubled the yield of *E. histolytica* obtained by the direct fecal films, significantly increased the yield of protozoa, and recovered approximately 80 per cent of the helminths.

(6) Brine centrifugal floatation shrunk protozoan cysts so badly that they were undiagnosable, although for helminths it approximated the efficiency of zinc sulphate.

(7) For *protozoa,* no combination of previously used diagnostic technics tested equalled the results obtained by the zinc sulphate technic alone. Except for the brine technic no combination reached 25 per cent of the yield of *helminths* obtained by the zinc sulphate technic in our lightly infected individuals.

(8) A combination of one of the direct iodine-stained or hematoxylin-stained fecal films with zinc sulphate centrifugal floatation, either by touch-removal or loop-removal, yielded 80 per cent for *Endamoeba histolytica,* 80 per cent for all protozoa and 85 per cent for all helminths found by all technics tested.

4. Because of simplicity in operation and high efficiency of yields for both intestinal protozoa and helminths, a combination of the direct fecal film (either iodine-stained or hematoxylin-stained) with zinc sulphate centrifugal floatation (either by touch-removal or, preferably, by loop-removal) is recommended for the clinical laboratory and in epidemiological surveys.

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TWO NEW SPECIES OF STRIGEID CERCARIAE IN LYMNAEID SNAILS FROM THE UNITED STATES[1]

STERLING BRACKETT

Many species of strigeid cercariae have been described from the Douglas Lake, Michigan, region (Cort and Brooks, 1928; Cort and Brackett, 1937, 1938a and b). During studies on cercariae in two other regions of the United States (Wisconsin and Louisiana) a number of these same cercariae were found. In addition, two which were not described in the above papers nor in any others as far as could be determined were observed and are characterized here. The name *Cercaria riponi* n. sp. is proposed for the form found in *Stagnicola palustris elodes* from Rush Lake a few miles north of Ripon, Wisconsin. For the other form which was found in *Fossaria parva* from Baton Rouge, Louisiana, in which the penetration glands are small and obscure, the name *Cercaria obscuradena* n. sp. is proposed. The methods used for the study of these cercariae have been described in another paper (Cort and Brackett, 1937).

Cercaria riponi n. sp.

(Fig. 1)

Specific diagnosis: Small strigeid cercaria with body wider but shorter than tail stem; furcae slightly longer than tail stem; body length[2] 127–207 μ, mean 146 ± 2.0 μ; distance from middle of ventral sucker to posterior end of body 54–81 μ, mean 63 ± 0.7 μ; body width 36–52 μ, mean 44 ± 0.5 μ; tail stem length 144–180 μ, mean 163 ± 0.8 μ; tail stem width 34–44 μ, mean 38 ± 0.3 μ; furcal length 144–189 μ, mean 169 ± 1.2 μ. Oral sucker longer than wide, length 28–44 μ, mean 35 ± 0.3 μ, width 22–28 μ, mean 24 ± 0.01 μ. Ventral sucker width 24–32 μ, mean 28 ± 0.15 μ. Prepharynx very short; pharynx width (21 measurements) 10–12 μ, mean 11 ± 0.08 μ; esophagus bifurcating about halfway between oral and ventral suckers; intestinal ceca moderately well developed, extending just slightly posterior to the ventral sucker. Two preacetabular bodies larger than the so-called "unpigmented eye-spots" of some cercariae but smaller than similar bodies described in *C. dohema* Cort and Brackett, 1937. No forward projecting spines at the anterior tip of the body; spines in oral cap not very large, irregularly arranged and extending back about half the length of the oral sucker; finer spines scattered thinly over body back of oral cap almost to ventral sucker; spines not noted on tail stem and furcae; three or four thick, irregular rows of spines encircling the ventral sucker. Penetration glands, finely granular, six in number and moderately large, occupying almost all of the space between the ventral sucker and the genital primordium; ducts of penetration glands not prominent except at their anterior ends and only slightly enlarged in passage through oral sucker. Genital primordium a prominent mass of cells in front of bladder. Tail stem with 12–14

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[1] A contribution from the Department of Zoology of the University of Wisconsin.

[2] Unless otherwise indicated the measurements are of 25 specimens. An analysis showed that a sample of 25 gave practically as good results statistically as a sample of 50 which was used in earlier descriptions. The extremes and the probable error of the mean are used to indicate the character of the variation.

large caudal bodies, the first 4 or 6 arranged in pairs with the rest forming a single row extending to posterior end; furcae as in other strigeid cercariae. Excretory system with bladder commissure at posterior margin of ventral sucker; two flame cells on each side connected with anterior and two pairs with posterior collecting tubules; two flame cells in proximal part of tail.

Daughter sporocysts with blunt, somewhat protrusible, anterior tip, short birth canal and prominent birth pore.

Host: Stagnicola palustris elodes (Say)

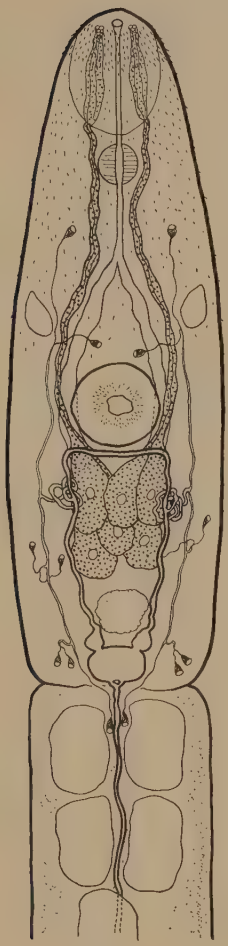
Locality: Rush Lake, Wisconsin, U.S.A.

Only one individual of 260 *S. palustris elodes* collected late in the summer of 1938 from a marshy stream adjoining Rush Lake was found infected with this species of cercaria. Snails collected from the same area early in the summer did not harbor this trematode.

When undisturbed, more individuals of this species of cercaria will be seen suspended motionless in the water than will be seen swimming. The body is downwards and is contracted so that it appears short and stubby; the furcae are held at right angles with the tail stem making a perfect figure "T". Most but not all of the hanging individuals are in a vertical position. They tend to go upwards when swimming but since they often change their course their path may take a lateral or even downward direction. In undisturbed water the distance travelled during each period of activity is not great, being only five to ten times the length of the body but in agitated water they will swim much farther. A tendency to collect on the side of the container away from the highest light intensity was noted. Vertical distribution was quite uniform with the exception that very few cercariae were seen in the uppermost or lowermost centimeter of water. The time of emergnce was not definitely noted but a great number of individuals appeared between 5:00 PM of one day and 9:00 AM of the next.

The number of described species of strigeid cercariae which possess six penetration glands posterior to the ventral sucker is very limited. Of these, Cercaria I Petersen 1931 from lymnaeid snails is most like *C. riponi*. Neither Petersen's description nor diagram are completely adequate for comparison with later work. Wesenberg-Lund (1934) refers a strigeid cercaria found in lymnaeid snails in Denmark to this species of Petersen's. This is a more detailed description and includes measurements. Petersen figures no caudal bodies but this may be due to the fact that his specimens were taken directly from snail tissue before they emerged naturally. The longer intestinal ceca of the cercariae of both of these workers differs from the shorter ones of *C. riponi*. Using Wesenberg-Lund's description the following differences between *C. riponi* and Cercaria I Petersen can be noted. *C. riponi* has fewer flame cells in both body and tail, fewer caudal bodies, the ventral sucker is more heavily spined and possesses a distinct pair of "preacetabular bodies."

The morphological features of this strigeid cercaria useful for diagnosis are indicated in the specific diagnosis and the drawing. An addi-



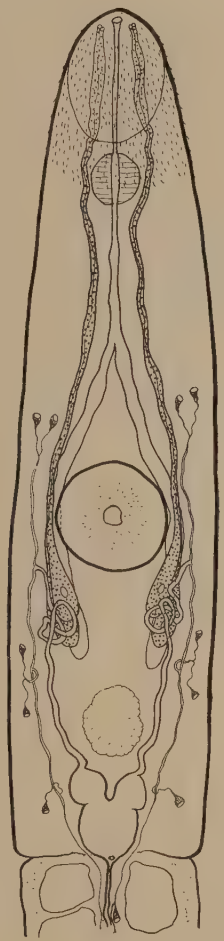


FIG. 1. Semidiagrammatic drawing of the body of *C. riponi* showing structural details, ventral view.

FIG. 2. Semidiagrammatic drawing of the body of *C. obscuradena* showing structural details, ventral view.

tional and unusual structure was noted in cercariae that had not yet emerged from the snail tissue but were otherwise fully grown. A pair of pores was present on either side of the ventral surface about half the length of the oral sucker back from the anterior end. Leading away from these pores (four in all) were ducts containing a coarsely granular

substance and otherwise very similar in appearance to the ducts of the penetration glands. They could be traced as a pair of ducts on each side of the body extending posteriorly a short distance back of the pharynx where they became lost to view. No evidence of any glands to which they might have been related was noted although there was sufficient room in front of the ventral sucker for such structures. Of particular interest in this connection is a structure described and figured by Petersen in his Furcocercaria I (Wesenberg-Lund's Cercaria I Petersen) as "ein quergelagerter Zellhaufen ohne Ausführgänge vor dem Bauchsaugnapf." The diagram of his Furcocercaria II, another strigeid with six glands back of the ventral sucker, also possesses a similar mass of cells labelled "Drusenzellen." Nothing can be said by the writer at this time about the nature of the unique structure described by Petersen or of the unusual set of pores and ducts in *C. riponi* but the fact that these occur in what appear to be closely related species may be of some significance.

The daughter sporocysts of *C. riponi* are threaded in and out of the liver of the snail host and complete specimens are practically impossible to obtain. The longest fragment measured was 5.7 mm in length and about 0.14 mm in diameter. Only old specimens were found but they were not heavily pigmented. The protrusible tip is quite short and often not extended giving the anterior end a rounded appearance. The birth pore is very prominent. The ridge around the protrusible tip is difficult to see.

Cercaria obscuradena n. sp.

(Fig. 2)

Specific diagnosis: Strigeid cercaria with body about the same width but much longer than the tail stem; furcae longer than tail stem and almost as long as body; body length 169–221 μ, mean 191 ± 1.9 μ; distance from middle of ventral sucker to posterior end of body 71–97 μ, mean 85 ± 1.0 μ; body width 35–54 μ, mean 45 ± 0.7 μ; tail stem length 110–136 μ, mean 124 ± 1.2 μ; tail stem width 32–38 μ, mean 36 ± 0.4 μ; furcal length 143–195 μ, mean 176 ± 1.7 μ. Oral sucker almost as wide as long, length 29–38 μ, mean 33 ± 0.4 μ, width 26–32 μ, mean 29 ± 0.2 μ. Ventral sucker width 26–32 μ, mean 30 ± 0.2 μ. Prepharynx very short so that pharynx almost touches the oral sucker; pharynx (9 measurements) 13–16 μ, mean 14 μ; esophagus bifurcates about halfway between oral and ventral sucker; intestinal ceca well developed but slender and extending only half the distance from the ventral sucker to the posterior end. "Unpigmented eyespots" not seen. No forward projecting spines at the anterior tip in the circumoral spineless area; no oral cap of heavy spines but there are fine spines scattered rather thickly over the anterior tip of the body and extending back slightly beyond the posterior margin of the oral sucker; rest of body without spines except for about four circles of small spines encircling the ventral sucker. Penetration glands four in number and situated behind the ventral sucker but very obscure and difficult to see and not readily stained with nile-blue sulfate; ducts of the penetration glands quite prominent in their anterior extention but do not expand noticeably in the oral sucker. Genital primordium a rather large mass of cells in front of bladder. Tail stem with 16–20 caudal bodies that are not very prominent and are not arranged regularly in pairs; furcae as in other strigeids; excretory system without bladder commissure; two flame cells on each side connected with anterior and three con-

nected with posterior collecting tubules; no cilia were seen within the tubules; two pairs of flame cells in proximal part of tail.

Daughter sporocysts not studied in detail but the birth pore and the ridge around the anterior tip are both readily seen. The cercariae are packed in the sporocysts irregularly and do not necessarily lie parallel.

Host: Fossaria parva (Lea).

Locality: Lake Content, a short distance north of Baton Rouge, Louisiana, U.S.A.

This cercaria was first found in a fairly high percentage of snails collected on Dec. 18, 1937. A second collection of 327 snails was made on Dec. 22. These were all crushed and 66 or 20% were found infected with this cercaria. On Dec. 28 a third collection of 479 was made at which time only 9 or less than 2% were infected. No explanation for this tremendous drop in the incidence of the infection can be given. The snails were collected from the same area, were the same in size, and in every other obvious way this third collection appeared to be identical with the second. Some snails collected from the same area in the spring of 1938 and sent to the author in Wisconsin were found to be uninfected with this species of cercaria.

This snail host, being very small, was isolated in stender dishes instead of milk bottles and the presence of cercariae noted by observing the water contained in the dishes from above through a binocular microscope. Observations of the activity of the cercariae made from this angle cannot be compared readily with those made from the side as has been done for the more recently described cercariae (Cort and Brackett and *C. riponi*). They were, however, observed to swim almost constantly in a manner similar to that described for *C. dohema* and the cercaria of *Cotylurus flabelliformis*.

Two cercariae previously described, *C. micromorpha* Brown 1926 (from *Limnaea peregra*) and *C. furcicauda* Faust 1919 (from *Anculusa carinata*) are similar in structure and are apparently closely related to *C. obscuradena*. The penetration glands of *C. obscuradena* are smaller than those of the two mentioned above. There are 16 or more caudal bodies in *C. obscuradena* as compared with 12 in *C. micromorpha*. In addition this cercaria differs from *C. furcicauda* in having a distinctly smaller body and shorter tail stem, shorter intestinal ceca, 4 instead of 2 flame cells in tail stem; and it differs from *C. micromorpha* in having 5 instead of 4 flame cells on each side of the body.

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THE MORPHOLOGY AND LIFE HISTORY OF *PETASIGER NITIDUS* LINTON (TREMATODA: ECHINOSTOMIDAE)[1]

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During the summer of 1938 the author was engaged in studies on the echinostome cercariae in the region of Douglas Lake, Michigan. One of the more common cercariae studied there was an undescribed species of the large-tailed type. Because of its conspicuous large tail and its fish-lure type of locomotion, large numbers of this cercaria were fed to several species of small fish as a first step in the determination of its life history. Suitable hosts for the metacercariae soon were determined and near the end of the summer adult worms of a previously described species, *Petasiger nitidus* Linton, 1928, were recovered from experimentally infected canaries.

THE CERCARIA

(Figs. 5–9)

The most common host for the cercaria in the region of Douglas Lake is *Helisoma antrosum percarinatum* (Walker), ten per cent of which were infected in some collections. It occasionally was found in *H. campanulatum smithii* (Baker). The cercariae emerge from the snail in fairly large numbers. A single snail may produce 500 to 1,000 cercariae in the first two or three days after reaching the laboratory, and subsequently liberate from 50 to several hundreds daily for two weeks or longer. Emergence is during the day, mostly before mid-morning. Immediately after emergence there is a period of creeping and inactivity followed by a period of about 36 hours of alternately swimming to the surface and passively sinking. While swimming, the body is greatly contracted and slightly flexed. The powerful tail usually precedes the body, sculling somewhat lazily upwards in a serpentine fashion which forms a continuous "S" instead of the more familiar "8" seen in other types of echinostome cerceriae. Due to pronounced positive reaction to light, they readily become concentrated. The tail will continue to swim if forcibly detached but under usual conditions the tail and body become spent and die together.

Description. Living cercaria colorless. Total length 1 mm to 1.4 mm with tail contributing from eight-tenths to nine-tenths of the length. Contracted, the

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body is nearly round with a diameter of around 0.13 mm; fully extended it is 0.22 mm by 0.05 mm. Shape typical of echinostomes, with crown area distinct, acetabulum in posterior half of body and at level of greatest width. Cuticula beset with minute spines, more conspicuous anteriorly. Crown spines 19, arranged in a single unbroken dorsal row of 11 that are equal in size, and 4 larger ones on each of the ventral angles. Length of dorsal spines 5 μ to 6 μ; angle spines 7 μ to 8 μ. Oral sucker and acetabulum of nearly equal size; diameter 26 μ to 32 μ in fixed specimens which measure 0.139 mm by 0.06 mm to 0.18 mm by 0.054 mm. Prepharynx long; pharynx small and oval, 6 μ by 8 μ. Esophagus and crura poorly developed but may be traced after staining. Three conspicuous gland cells are located on either side of the upper esophagus. Pharynx and prepharynx always contain a deep brown pigment in the lumen, and sometimes the upper esophagus contains some of the same. Excretory bladder of two compartments with the anterior one deeply bi-lobed and often appearing as furci. Excretory tubes of three longitudinal loops: first, an ascending loop, the siphon, extending from bladder to oral sucker and containing 20 to 25 irregular granules 10 μ to 12 μ in diameter which appear to have been formed by fusion of 2 or 3 smaller spheres; second, a descending loop extending unbranched to level of bladder where it turns anteriorly to form the third loop. The latter sends branches to 3 groups of 3 flame cells each in the areas around bladder, acetabulum, and oral sucker respectively. Flame cell formula 2(3+3+3). Most of body back of pharynx filled with irregularly placed rectangular bars of cystogenous material. Each unit appears to contain numerous minute colorless bars. Base of tail variable in shape, somewhat distinct from remainder, and contains a large central vesicle; the base nearly as wide as remainder of tail when contracted but very narrow and attenuated when extended. Rest of tail very slightly flattened dorso-ventrally, blade-shaped in profile. Circular muscles well developed throughout length of tail; longitudinal bands central and poorly developed; circulo-diagonal bands well developed in the blade but not in the basal portion.

THE REDIA

(Figs. 11–13)

Description. The rediae are long, slender, and very motile. Color yellowish to brown, not orange or reddish. Heavier infections have rediae of all sizes from 0.15 mm to nearly 2 mm in length. Posterior appendages and collar well developed. Collar divided into four distinct folds located on the dorsal, ventral, and lateral surfaces near the anterior end of the body. Birth pore dorsal, and in mature rediae it protrudes considerably above the body surface immediately posterior to the collar. Gut extends to level of posterior appendages and usually is filled with brown or black granules. Flame cells arranged in 4 groups, 2 back of collar and 2 near appendages. The smallest number observed in each group was 12; accurate counts were impossible in the largest rediae but there are at least 40 in each group. Cercariae in all stages of development occur in each of the older rediae. Although the cercariae seem to be fully developed in the rediae, they apparently require a period of maturing in the snail's tissues. Great masses of cercariae consistently were found free in the tissues mostly concentrated in the center of the digestive gland along its duct. The following measurements were taken from fixed material. Length and width of large extended specimen, 2.5 mm by 0.18 mm; contracted specimen of about equal size, 1.7 mm by 0.24 mm. Average specimen in moderate contraction, 1.2 mm by 0.15 mm; pharynx 48 μ by 44 μ; from anterior end to collar, 0.187 mm; from collar to posterior appendages, 0.69 mm.

THE METACERCARIA

(Fig. 10)

When the cercariae are eaten by small fish, both the body and tail are taken into the stomach. Here the body becomes detached and migrates

forward to the esophagus and lower pharynx where encystment occurs. To accomplish this the cercariae penetrate the mucosa, enter the lamina propria, and form a double cyst wall 3 μ to 4 μ thick. After about 4 days the host will have added several irregular fibrous layers to the cyst and in the course of 10 days a heavy outer wall 8 to 10 times thicker than the original is formed. The shape of the cyst is almost invariably ovoid with the sides slightly flattened. Exclusive of host tissue the cysts vary around 85 μ by 68 μ. Only a few changes are noticeable in the metacercariae after encystment. When it becomes infective (7 to 9 days) the oral and cuticular spines are more fully developed. The upper part of the esophagus forms a lumen and becomes filled with the brown pigment earlier confined to the pharynx and prepharynx. The large irregular excretory granules which were formerly confined to the preacetabular region gradually extend more and more posteriorly until they reach the bladder.

By isolating them in small dishes with known numbers of cercariae the following fish were experimentally infected: *Ameiurus nebulosus, Notropis hudsonius, Umbra limi, Lepomis pallidus, Ambloplites rupestris, Perca flavescens, Lebistes reticulatus,* and a number of undetermined "minnows."

THE ADULT

(Figs. 1–4)

Fish carrying infections of metacercariae were fed to 6 pigeons, 4 canaries, 1 rat, 2 mice, 1 cat, and 1 duck. Two of the canaries produced adult worms. The first was given 250 to 300 metacercariae which were 11 days old, and after 7 days 181 slightly immature worms were recovered. The second was given 80 to 100 metacercariae which were 7 and 9 days old. Ova appeared in the stools on the ninth day and were very abundant on the tenth and eleventh days. On the twelfth day an entire worm (living) was found in the feces and by immediate autopsy 10 mature specimens were taken from the duodenum. The failure of this worm to develop in the other hosts may have been due to immaturity of the metacercariae. In each case they were less than 5 days old.

Although the adult has been described briefly by Linton (1928), it seems advisable to restate the diagnostic characters and add certain observations that may be essential to systematic studies on this and related species. In the following description Linton's observations are taken into account, but all statements regarding morphology are based on a study of specimens taken from the duodenum of experimental canaries. Linton's collection came from the Horned Grebe (*Colymbus auritus*) from the region of Woods Hole, Massachusetts.

Description. Small, fusiform, 1.26 mm to 2.25 mm in length. Crown conical to reniform, bearing 19 relatively large spines; 11 of crown spines equally spaced

in a single unbroken dorsal row, and 4 somewhat larger ones located in a compact group on each ventral angle of the crown; the 2 more superficial or more anterior ones being slightly smaller than the other two. Body spination total in small worms but reduced to anterior end in larger ones. Body compressed dorsoventrally in anterior region, round in cross section posterior to acetabulum. Acetabulum in middle of body, nearly three times larger than oral sucker. Oral sucker nearly terminal, pyriform to spherical. Prepharynx of moderate length; pharynx oval to pyriform. Esophagus extends to genital pore and crura end halfway between posterior testis and tip of body. Genital pore median or slightly to left; genital atrium deep and with thick wall. Two testes contiguous, crowded into oblique position with the anterior one more to the left. Cirrus sac large, somewhat dextral, posterior border near the middle of acetabulum; anteriorly it reaches far beyond acetabulum to crowd the gut bifurcation. Seminal vesicle moderately developed, usually with two compartments. Pars prostatica of moderate length; prostate gland very massive, occupying two-thirds or more of cirrus sac. Cirrus simple, with musculature well developed. Ovary dorsal and to right of mid-line. Oviduct very short, extending dorso-mesially to mid-line where it receives Laurer's canal and the common yolk duct just before entering the shell gland. Shell gland large, median, and dorsal to anterior testis. Uterus very short, forming one to three loops between genital complex and acetabulum. Ova few; usually less than 6 in specimens under 2 mm in length. Metraterm short; situated to right of cirrus sac and emptying into genital atrium in front of cirrus. Vitelline reservoir median, dorsal to posterior border of anterior testis. Vitelline follicles extend from just anterior to acetabulum along sides of body to posterior end where the two lateral fields are joined posterior to hind testis and dorsal to bladder. Seminal receptable absent. Measurements on a single typical worm selected from a collection of 12 day old specimens from a canary: length, 1.26 mm; width at acetabulum, 0.426 mm; width at crown, 0.22 mm; at neck, 0.187 mm; preacetabular length, 0.52 mm; postacetabular length, 0.53 mm; acetabulum, 0.232 mm long by 0.24 mm wide; oral sucker, 0.08 mm long by 0.09 mm wide; diameter of pharynx 0.067 mm; cirrus sac, 0.258 by 0.159 mm; anterior testis, 0.19 mm by 0.16 mm; posterior testis, 0.22 mm by 0.14 mm; diameter of ovary, 0.12 mm; dorsal spines, 78 μ by 14 μ; largest angle spine, 90 μ by 16 μ; longest preacetabular body spines, 15 μ; ova vary around 90 μ by 60 μ.

Linton's description was made from a collection of larger worms. His measurements give similar proportions, however. One important correction should be noted. This species does not possess a true seminal receptacle as described and illustrated by Linton. It has instead a seminis receptaculum uterinum.

DISCUSSION

There are four other described species of large-tailed echinostome cercariae, all of which closely resemble the cercaria of *P. nitidus*. They are *Cercaria magnacauda* O'Roke, 1917; *C. caudadena* Faust, 1921; *C. cita* Miller, 1925; and *C. oscillatoria* Brown, 1931. The description of *C. magnacauda* includes the following characters which were not observed in the cercaria of *P. nitidus*: it creeps only when the tail is lost; the tail becomes detached and continues swimming after the death of the body; there is a synchronous activity in the swarms of the cercariae; the redia has a short gut and a large median oral stylet; the diameter of the acetabulum is two times that of the oral sucker; and its host is *Helisoma trivolvis* in Kansas. *C. caudadena* is slightly larger than the cercaria of

P. nitidus and further differs from it in that the crura of the gut are much shorter, the crown spines are more numerous (? not more than 24), and the tail contains a central core of large spherical glandular cells. It was discovered in *Planorbis pfeifferi* from Merebank, South Africa. *C. cita* was discovered in *Planorbis altissimus, P. operculatus* and *P. vermicularis* from San Juan Island, Puget Sound, Washington (Miller, 1929). It is a smaller species which does not have brown granules in the pharynx and prepharynx; its cystogenous glands are yellowish; its collar spines are too small to be clearly discerned; its redia does not have well formed appendages; and the developing cercariae possess exactly 6 large brown pigmented cells in the tail. *C. oscillatoria* develops in *Planorbis carinatus* in Cheshire, England. Apparently it differs from the cercaria of *P. nitidus* in having suckers and pharynx nearly two times larger; in having the collar spines less developed; in oscillating as it falls through the water instead of holding its tail straight; and in having brown pigment in the tissues lateral to, rather than within the prepharynx.

While it would seem that there are a number of differences between the 5 species under consideration, it is doubtful if all of those mentioned above are actually real differences. The variation in behavior may be due to diversity of the respective unnatural conditions under which they were observed; structural characters allow diverse interpretations, *e.g.*, the crown spines may seem well developed or poorly developed depending upon technics employed and opportunity for re-examination of abundant material; and the exact size of such an organism is difficult to determine. If they are all distinct species, they doubtless constitute a homogeneous group of echinostome cercariae with similar life histories. Brown (1931) found cysts in the tissues of the cercarial host snail and tentatively described them as metacercariae of *C. oscillatoria*. He probably observed there another species of echinostome.

Complete or nearly complete life histories are known for about 18 species of the Echinostomidae. The majority of them fall into a group in which the cercariae are rapid swimmers and migrate without much penetration into either snails or larval frogs (or both), where they form a heavy-walled cyst and are infective almost as soon as encystment is accomplished. Examples of this type are *Echinostoma revolutum* (Johnson, 1920, and Beaver, 1937) and *Echinoparyphium recurvatum* (Mathias, 1927, and Rasin, 1933). A less common type is illustrated by *Parorchis avitus* in which the cercaria encysts in the open (Stunkard and Cable, 1932). The life history of *Petasiger nitidus* more closely resembles a third type in which the cercaria more passively reaches its site of encystment, being eaten by fish or carried to their gills by respiratory water currents. Within the host this type of cercaria actively penetrates the tissues of the pharynx, gills, or esophagus and forms an oval heavy-

walled cyst. In addition to *P. nitidus, Echinochasmus perfoliatus* as demonstrated by Muto (1921) is also an example of this type.

Thus it is possible to recognize "types" of life histories for the ECHINOSTOMIDAE. Because some of the data are contradictory and some need confirmation, and because no information is available on many of the more unique genera, it seems inadvisable to suggest alterations in our present classification which makes use of three subfamilies (ECHINOSTOMINAE, HIMASTHLINAE, and ECHINOCHASMINAE) to receive most of the genera, but leaves a dozen others including *Petasiger* in an isolated position. The need for further studies on the ECHINOSTOMIDAE is evident.

SUMMARY

1. A new large-tailed echinostome cercaria is described from *Helisoma antrosum percarinatum* from the region of Douglas Lake in the northern part of the southern peninsula of Michigan.

2. The metacercarial stage is described and a number of minnows are established as second intermediate hosts.

3. The canary is established as a laboratory host for the adult stage.

4. The adult worm is identified as *Petasiger nitidus* Linton, 1928, and the species redescribed.

5. Related cercariae and the types of echinostome life histories are discussed.

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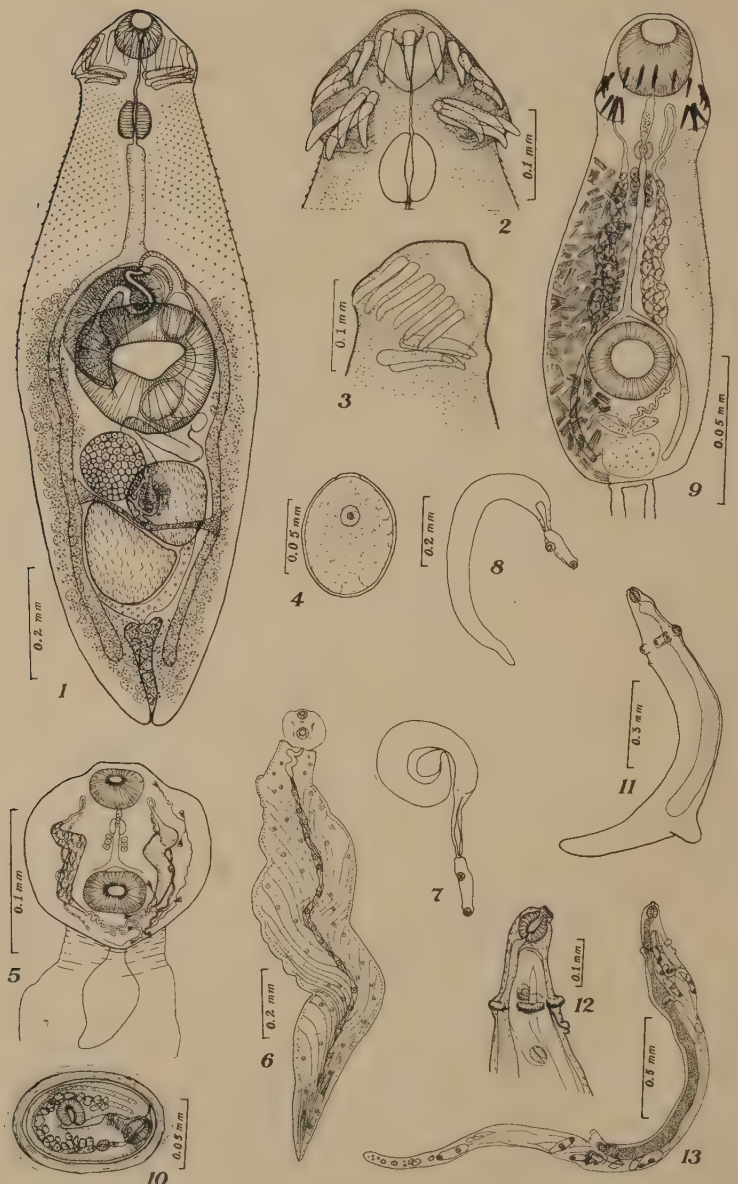
EXPLANATION OF PLATE P. 276

Drawings made with the aid of a camera lucida.

1. Young adult from experimental canary. Ventral view.
2. Head crown of adult. Frontal view.
3. Head and crown of adult. Lateral view.
4. Egg.
5. Cercaria. Ventral view of living specimen (body and base of tail) under pressure of cover glass, showing details of excretory system.
6. Cercaria. Ventral view of entire living specimen under pressure of cover glass.
7 and 8. Cercariae showing typical attitudes of the body and tail. (Drawn from fixed material.)
9. Cercaria. Ventral view of body moderately extended. (Drawn from fixed specimen.)
10. Metacercaria. (Drawn from living specimen.)
11. Redia. Lateral view showing relationship of main structures in a contracted specimen of average size. Developing cercariae omitted.
12. Redia. Lateral view of anterior end showing relationship of collar, birth pore, gut, and pharynx.
13. Redia. Large extended specimen.



Petasiger nitidus

RESEARCH NOTES

A NEW SPECIES OF *BRACHYLAEMUS* FROM THE BARRED OWL

In January 1938 a barred owl, *Strix varia varia,* was examined for parasites. From the small intestine 62 flukes were obtained. Mr. Allen McIntosh of the Zoological Division, U. S. Bureau of Animal Industry, diagnosed them as a new species of *Brachylaemus* for which the name *Brachylaemus mcintoshi* is proposed.

Brachylaemus mcintoshi n. sp.

Specific diagnosis: Brachylaemus; Family Brachylaemidae.—Body cylindrical 2.6 mm long by 0.29 mm wide; weakly spinose. Oral sucker subterminal, well developed, 210 μ long by 163 μ wide; oral opening longitudinal. Ventral sucker smaller than oral sucker being 130 μ long by 121 μ wide, dividing body approximately 1: 3.5. Pharynx 77 μ × 95 μ. Esophagus short. Intestinal ceca extend to near posterior extremity of body. Reproductive organs median, contiguous and tandem, in posterior region of body. Anterior testis 164 μ × 153 μ; posterior testis 164 μ × 144 μ. Seminal vesicle runs forward in front of anterior testis for a distance of 190 μ; cirrus enters genital atrium from left side, occasionally protruding through pore. Genital pore median, just anterior to anterior testis. Ovary 120 μ × 124 μ, between testes. Oviduct arises from median posterior portion of ovary. Mehlis' gland and vitelline reservoir ventral to ovary, inconspicuous; fecundarium antero-lateral to posterior testis. Laurer's canal dorsal to posterior margin of ovary. Vitellaria extracecal beginning approximately at level of genital pore; anterior extent variable, usually at level of ventral sucker, seldom anterior or posterior to it (Type, Fig. 1, illustrates both extremes).

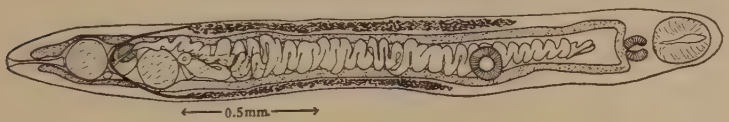


Fig. 1. *Brachylaemus mcintoshi* n. sp., ventral view.

Vitelline ducts unite postero-ventral to ovary. Metraterm enters genital atrium from right side. Uterus filled with eggs, more or less intercecal extending 320 μ beyond the ventral sucker. Eggs brownish yellow 30 μ × 19 μ. Excretory bladder cone-shaped giving off two primary branches which extend to region of pharynx and then bend sharply posteriad.

Host: Strix varia varia Barton.

Habitat: Small intestine.

Locality: Raleigh, North Carolina.

Specimens: U. S. Nat. Mus. Helm. Coll. Nos. 43370 (Type) and 43371 (Paratype).

No other species of *Brachylaemus* with oral sucker larger than the ventral sucker has been described from North American birds. There are seven species with oral sucker larger than ventral sucker found in birds. These are *B. mordens, B. centrodes, B. commutatus, B. fuscatus, B. mazzantii,* and *B. pulchellus. Brachylaemus mcintoshi* n. sp. can be separated from these very easily. A round mouth opening is present in *B. mordens, B. mazzantii* and *B. pulchellus.* The reproductive organs are either not strictly tandem or not contiguous in all these except *B. mazzantii.* In the latter species the vitellaria extend almost to the region of pharynx. All of the above species are larger than *B. mcintoshi.*—Reinard Harkema, *North Carolina State College, Raleigh, N. C.*

PRELIMINARY NOTE ON THE OCCURRENCE OF LEUCOCYTOZOON *SMITHI* LAVERAN AND LUCET (1905) IN TURKEYS IN THE SOUTHEASTERN UNITED STATES

Leucocytozoon smithi was found in blood smears from a sick domestic turkey in a plantation near Newton, Ga.

Since Simuliidae are reported to transmit this disease in other sections of the United States, and in view of the scarcity of these insects in south Georgia, a survey was made to determine the incidence of the disease in relation to other possible insect vectors. A random sample of turkeys from Georgia and Florida was examined for the presence of the parasite, and blood smears from birds secured from various points in Alabama and South Carolina were also secured. Through the courtesy of H. L. Stoddard of the Cooperative Quail Study Association an opportunity was afforded to examine blood specimens from captive wild turkeys in Missouri.

A large percentage of the samples in Georgia were taken from turkeys in poultry markets; but in Florida most of the specimens were obtained directly from the producer. Through the cooperation of local sportsmen, 30 wild turkeys were examined, 5 from Georgia and 3 from Florida. All these wild turkeys were infected with *L. smithi*. Apparently this parasite has not been previously reported from turkeys in the wild. However, Johnson et al. (1938, Am. J. Hyg. 27: 648–649) reported it from captive wild turkeys in Virginia. The number of infected birds is shown in Table 1. Our finding of this disease in Alabama

TABLE 1.—*The number and age of domesticated turkeys found infected with Leucocytozoon smithi in Missouri, Georgia, Florida, Alabama and South Carolina in 1947*

State	Stage of development of turkeys and manner infected						Total number examined
	Adult		Young		Age undetermined		
	Infected	Not infected	Infected	Not infected	Infected	Not infected	
Georgia	280	46	0	12	1	5	351
Florida	48	4	0	1	0	1	54
Missouri*	7	14	0	1	0	1	23
Alabama	4	3	0	1	0	1	9
South Carolina	2	2	0	0	0	1	5
Total	341	79	0	14	1	9	444

* Captive wild birds.

Georgia, South Carolina, Florida and Missouri is believed to represent new locality records for this parasite in domestic turkeys.

Thirteen pheasants, guinea fowl and ducks were examined on farms where there were infected turkeys, but no leucocytozoa were found in these birds.

Although sufficient data were not obtained in the survey to indicate definitely which species of insects were responsible for the transmission of the disease in the Southeast, it was noted that in low areas in Georgia and Florida where wooded marshes and ponds were numerous nearly 100 per cent of the adult turkeys were infected with *Leucocytozoon smithi*. This suggests that some species breeding there is involved.—Benjamin G. Travis, *Division of Insects Affecting Man and Animals, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, M. H. Haskins, Jr., Malaria Research Foundation, Thomasville, Ga.* AND E. GAMBRELL, *Emory University, Atlanta, Ga.*

STAINING MALARIA OOCYSTS IN LIVING MOSQUITOES

We have found it possible to stain oocysts of *P. cathemerium* in *A. maculipennis* [illegible] by feeding the mosquitoes on a 10 per cent glucose solution to which has

been added a small quantity of eosine (water-soluble). In mosquitoes which fed on this solution for two days the oocysts were clearly stained and so more readily seen on dissection of the mid-gut. Sporozoites were not stained with eosine.—P. F. Russell and B. N. Mohan, *King Institute, Guindy, Madras.*

SCHIZOTAENIASIS IN MUSKRATS

Four hundred and fifteen anoplocephaline tapeworms of the genus *Schizotaenia* were collected from a formalin-preserved gastro-intestinal tract of one of four muskrats (*Ondatra zibethica zibethica* Linn.) taken near Ely, Minnesota by Mr. Shaler E. Aldous, U. S. Biological Survey.

Upon examination of this particular specimen of muskrat, the stomach was found to be almost full of small, stout tapeworms, many of which are sexually mature, and all in an excellent state of preservation. They are obviously of two types, one very broad and the other with less than half the breadth of the former. Examination of whole mounts and sections showed them to be *Schizotaenia variabilis* Douthitt, 1915, 80 specimens, and *S. americana* (Stiles, 1895), 355 specimens. Both of these cestodes appear simultaneously in the porcupine, *Erethizon dorsatum dorsatum* (Linn.) from northern Minnesota (Jellison, 1933, Tr. Am. Micr. Soc. **52**: 42–47) and in *E. epixanthum epixanthum* Brandt from Colorado (Chandler, 1936, J. Parasitol. **22**: 513).

The specimens from the porcupine attain a much greater size than those from the muskrat. The largest specimen of *S. americana* from the muskrat measured 22 mm, while the largest specimen from the porcupine measured from the many individuals in the collection reached 168 mm. Stiles (1895, Vet. Mag. **2**: 341–346) described *S. americana* as being 33 mm long. Similarly with *S. variabilis*, large specimens from the muskrat are considerably smaller than the smallest ones from the porcupine, while the large porcupine specimens are many times the size of the muskrat specimens. Strobilae sizes, however, are so variable that they have little specific significance when considered alone. Measurements of the scolex, cirrus pouch, ovary and other organs, together with their relationships to each other, are in reasonably close agreement with those of the porcupine specimens as given by Stiles (1895) and Douthitt (1915).

Jellison (l. c.) reported 358 scolices of various sized cestodes taken from a single specimen of porcupine, *Erethizon dorsatum* from Pine County, Minnesota. Examination of Jellison's material, together with that from numerous subsequent collections, shows both *Schizotaenia americana* and *S. variabilis* to be simultaneously present in the same host. Chandler (l. c.) pointed out the obvious differences in the size and shape of these two species of tapeworms and stated that they are widely distributed, occurring simultaneously in the two American porcupines, *E. dorsatum* and *E. epixanthum*. *Schizotaenia americana* is the smaller, being slender and having the female genital glands placed near the median line so as to appear more or less in a straight row while *S. variabilis* is considerably broader and with the female glands much nearer the poral margin of their respective proglottids thus forming what appears to be a double row of female reproductive organs instead of the single median one.

Of a total of several hundred Minnesota muskrats examined for parasites at this laboratory and by Mr. Lawrence Penner of the Zoology Department, this is the first instance of *Schizotaenia* occurring. Harkema (1936, Ecol. Monogr. **6**: 151–232) in his review of the parasites of rodents does not list it as occurring in muskrats. It appears to be a new host record. It might be pointed out that while several hundred muskrats have been examined for parasites without finding these cestodes, the great majority of them have originated from areas where the porcupine is not found commonly today. This condition suggests that the muskrat is an accidental rather than a normal host to *Schizotaenia americana* and *S. variabilis*.—O. Wilford Olsen, *Paper No. 1663 of the Scientific Journal Series of the Minnesota Agricultural Station, St. Paul. Also in cooperation with the Minnesota Conservation Department, Division of Game and Fish.*

THE PREPARATION OF A RELATIVELY STABLE UREASE CONCENTRATE FROM BLOWFLY LARVAE

Recently some investigators (Brown, 1938, Biochem. J. **32**: 903; Robinson and Baker, 1939, J. Parasitol. **25**: 149) have been concerned with an ureolytic enzyme of the blowfly, *Lucilia sericata* Meigen. Its concentration seems to vary with the age and stage of the insect. The amount of this enzyme in a given weight of fresh insect material may differ somewhat between any two lots of fresh specimens that appear to be of the same physiological age. Therefore, in order to investigate this urease in a reliable quantitative manner, it would seem very desirable to have a dependable source of the enzyme on hand at all times. However, this urease is somewhat unstable, and no little difficulty has been experienced in efforts to remove and preserve a constant and stable supply.

The writer has seen no description in literature of a successful attempt to remove this ferment from the living insects and to preserve it without a rather prompt and severe loss of activity. It, therefore, was thought advisable to record one method that has been fairly satisfactory.

The fully mature blowfly maggots are allowed to migrate from their food into a surrounding mote of clean sand. After they have burrowed about until free from food debris, the sand is removed from them by sifting it through a culinary strainer. The larvae that alone remain in the wire sieve after the sifting, are washed under a cold water tap from one to three minutes. The strainer then is emptied on one end of a heavy bath towel. Immediately, that end of the towel is rolled toward the opposite end with frequent in-foldings of the sides. Thus the maggots are confined within. As a result of their own agitation, they are soon dried and ready for the next step. (If necessary, they can be retained in the towel for a few hours by placing it in a refrigerator that has a temperature of about 5° C.)

The larvae are shaken from the towel into a mortar and crushed with a pestle until no intact specimens remain. The mortar containing the brei thus formed is placed in a vacuum oven surrounded by an oil bath that is held at a temperature of 35° C. A high degree of evacuation is maintained until all of the moisture has been removed. The process can be hastened somewhat by opening the oven and stirring the maggot residue from time to time. When the material is dry, the pestle is introduced, and grinding is resumed. It is continued until the product resembles crushed nut meats in appearance.

In order to reduce the bulk of the original source of the enzyme and to make the resulting product into a friable hydrophilic powder that can be weighed accurately, most of the fat should be removed. Many of the common fat solvents (e.g., acetone) seem to greatly impair or completely inactivate this urease. Several solvents were tried: absolute alcohol, acetone, benzene, chloroform, dioxan, petroleum ether, and sulphuric ether. It was found that the use of petroleum ether resulted in a powder which retained a high degree of urease activity. Petroleum ether to the extent of three to five times the volume of the dried maggots is added. The whole is thoroughly stirred and immediately poured into a funnel containing a coarse filter paper. The residue is rinsed once or twice on the filter with fresh petroleum ether.

After draining, the filter paper is removed. The maggot powder then can be passed through soil sieves to secure the desired particle size. The coarse fraction can be returned to the mortar and pulverized further, if need be.

The urease powder is placed in wide-mouthed, glass-stoppered bottles. These are sealed in the vacuum oven, and a short evacuation is effected to withdraw any moisture that might have been absorbed during the refining of the residue. Air is returned to the vacuum oven through a calcium chloride tube. The stoppers are inserted tightly, and the product is stored in a refrigerator for future use.

The writer found that such a crude concentration of blowfly urease—prepared and stored as outlined above—retains a fairly constant and high degree of activity for several weeks.—Fred C. Baker, *Formerly of the Cornell University Department of Entomology, Ithaca, N. Y.*

A METHOD FOR MOUNTING ANOPHELINE EGGS

During a study of the Venezuelan species of anophelines, it was found necessary to keep samples of the eggs until some adults from the same lots of eggs emerged. The methods found in the literature proved to be unsuitable in that by them the more delicate parts of the ova of some *Nyssorhynchi* were damaged. From our experience, the following procedure emerged. As we find that eggs mounted according to this technique have kept in good shape for almost a year, it appears that the method may be of general interest.

The eggs are obtained by allowing females to oviposit on wet filter paper. A small triangular piece containing the eggs can be cut and held with forceps while the eggs are washed off with 5 per cent formalin onto a square of fine filter paper placed on a slide. Absorbent cotton may be better than filter paper for some species, but the latter gives a whiter background for reflected light. Two small strips of thin paper are placed on the sides of the square to support a cover slip and the excess liquid drawn off with a piece of dry filter paper held at the side so as not to disturb the eggs. The cover slip is put into position carefully so as to eliminate all air bubbles and is sealed with du Noyer's mixture (1918, C. R. Soc. Biol. **81**: 741). This mixture is prepared by boiling 20 grams of lanolin until dehydrated, i.e., until the foam disappears, and adding 80 grams of rosin to make a homogeneous paste which keeps well when stored in metal boxes. Care should be taken to run the melted mixture well in around the cover, as drying of the formalin will ruin the mount.

The air inside the floats of the eggs is slowly absorbed by the mounting fluid. The star-like structures made by the exochorion on the ventral surface of the egg in some *Nysorrhynchi,* disappear in about five days, but this period allows time for making drawings or photographs. The floats and collar-like structure found in some of the eggs are very well preserved. The color of the egg changes from black to brown, the embryo contracts and is seen in the center of the egg as an oval shaped, darker portion. Counts of the ridges on the floats, measurements and other studies are easily carried out on the mounted eggs.—ARNOLDO GABALDON, *División de Malariologia, Dirección de Salubridad Pública, Ministerio de Sanidad & Asistencia Social, Caracas, Venezuela.*

GOMPHUS SPICATUS HAGEN (ODONATA) A NEW INTERMEDIATE HOST FOR *PROSTHOGONIMUS MACRORCHIS* (TREMATODA)

Of six naiads of *Gomphus spicatus* Hagen, obtained from Lake Phalen, Ramsey County, Minnesota, June 4, 1936, two were dissected and found to contain fifteen and twenty-five cysts respectively of *Prosthogonimus.* These, together with the remaining naiads were fed to a laying hen on the following day. Twenty-two days later the hen was killed and in the oviduct were found twenty large specimens of *Prosthogonimus.* A mature egg in the oviduct contained on the surface of the yolk a partially disintegrated fluke.

This species of *Gomphus* is very common in the Northeastern United States and Canada: Professor E. M. Walker states (private communication) that at Toronto it is abundant. It is a mud dweller, in this respect differing from the habits of other dragonflies implicated as transmitters of *P. macrorchis.* In view of the fact that the adults emerge during the last of May and the first part of June, as is true also of the other second intermediate hosts, the precaution of keeping poultry fenced from the shores of lakes applies likewise to this species.

Ducks feeding in the mud, as they frequently do, would tend especially to obtain *Prosthogonimus* infection from *Gomphus.*

My special thanks are due to Dr. William A. Riley for laboratory facilities at the University of Minnesota, and to Professor E. M. Walker for the identification of the dragonfly hosts.—RALPH W. MACY, *College of St. Thomas, St. Paul, Minn.*

A METHOD FOR THE PRESERVATION OF VIABLE FORMS OF *TRYPANOSOMA CRUZI*

Recently, Hoare (1938, Tr. Roy. Soc. Trop. Med. and Hyg. **32**: 8) described the development of mammalian trypanosomes in the body cavity of the caterpillar of the bee-moth, *Galleria mellonella* (Lepidoptera: Pyralidae), pointing out that when *Trypanosoma cruzi* is injected into these caterpillars survival always occurs and is followed by the production of the metacyclic trypanosomes characteristically found in the insect vector of this parasite. This worker suggested that this fact be utilized when it is necessary to send forms of *T. cruzi* to other laboratories. This note reports another, simpler method for a short term preservation of the viable forms of *Trypanosoma cruzi*.

Infection with *T. cruzi* in the rat is characterized, as in many other animals, by a somatic as well as vascular phase. When young rats up to 20 days of age are infected intraperitoneally they suffer an extremely intense and fatal infection in contrast to the mild, symptomless infection of adult rats (Kolodny, 1939, Am. J. Hyg., **29**, Sec. C: 1, 13). Intensity of peripheral blood stream infection in young rats is paralleled by extensive somatic invasion, particularly of cardiac musculature and spleen. It has been found that when either of these organs is removed during the peak of the blood stream infection, or immediately after the death of the animal, the leishmaniform stage of this parasite will remain viable for 12 days, and occasionally longer, when kept under proper conditions.

The following protocol will indicate the various methods of treatment and their effectiveness. Cardiac and splenic tissues of heavily infected nursling rats were placed in ordinary "Wassermann" tubes containing 2.0 cc each of the following materials: physiological saline, normal rat serum, buffered Ringer's solution and buffered Locke's solution. Similar tissues were placed over calcium chloride, and, for purposes of control, some were left untreated. All tubes were sealed. One set of tubes was placed in the refrigerator (about 4° C) and another set was kept at room temperature (about 23° C). These preparations were subsequently tested at intervals by grinding the tissues without abrasives in 1.0 cc of fresh fluid. It was found that tissues left untreated or over calcium chloride lose their infectivity after 1 to 2 days. Tissues kept in physiological saline were infective for about 8 days, while tissues kept in normal rat serum retained their infectivity for about 10 days. Cardiac and splenic tissues kept in either buffered Ringer's or buffered Locke's solutions, however, always retained their infectivity for at least 12 days and not infrequently for as long as 15 days. No differences were noted between the preparations kept at refrigerator or room temperature. Similarly, thorough washing in buffered Locke's solution immediately after removal from the animal body to remove the extravasated blood was also without effect upon the period of viability.

It may therefore be concluded that the leishmaniform stage of *Trypanosoma cruzi* retains its viability for about 12 days when the infected organ is kept in a sealed tube of buffered Ringer's or Locke's solution at either refrigerator or room temperatures. It is suggested that this parasite may be sent from one laboratory to another in this form.—MAXWELL H. KOLODNY, *Deparment of Bacteriology, College of Physicians and Surgeons, Columbia University, New York.*